

Exhibit 91

Disruption of Iron Homeostasis in Mesothelial Cells after Talc Pleurodesis

Andrew J. Ghio¹, Joleen M. Soukup¹, Lisa A. Dailey¹, Judy H. Richards¹, Jennifer L. Turi², Elizabeth N. Pavlisko³, and Victor L. Roggli³

¹National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, North Carolina; and ²Department of Pediatrics, and ³Department of Pathology, Duke University Medical Center, Durham, North Carolina

The mechanism for biological effects after exposure to particles is incompletely understood. One postulate proposed to explain biological effects after exposure to particles involves altered iron homeostasis in the host. The fibro inflammatory properties of mineral oxide particles are exploited therapeutically with the instillation of massive quantities of talc into the pleural space, to provide sclerosis. We tested the postulates that (1) *in vitro* exposure to talc induces a disruption in iron homeostasis, oxidative stress, and a biological effect, and (2) talc pleurodesis in humans alters iron homeostasis. *In vitro* exposures of both mesothelial and airway epithelial cells to 100 µg/ml talc significantly increased iron importation and concentrations of the storage protein ferritin. Using dichlorodihydrofluorescein, exposure to talc was associated with a time dependent and concentration dependent generation of oxidants in both cell types. The expression of proinflammatory mediators was also increased after *in vitro* exposures of mesothelial and airway epithelial cells to talc. Relative to control lung tissue, lung tissue from patients treated with sclerodesis demonstrated an accumulation of iron and increased expression of iron related proteins, including ferritin, the importer divalent metal transport 1 and the exporter ferroportin 1. Talc was also observed to translocate to the parenchyma, and changes in iron homeostasis were focally distributed to sites of retention. We conclude that exposure to talc disrupts iron homeostasis, is associated with oxidative stress, and results in a biological effect (i.e., a fibro inflammatory response). Talc pleurodesis can function as a model of the human response to mineral oxide particle exposure, albeit a massive one.

Keywords: talc; iron; ferritin; pleurodesis; particulate matter

Humans are routinely exposed to the particulate matter (PM) included in air pollution, cigarette smoke, environmental tobacco smoke, forest fires, gas and wood stoves, and the burning of biomass other than wood, as well as those particles contacted during the mining and processing of coal and mineral oxides. Many of the major global causes of death reported by the World Health Organization (<http://www.who.int/mediacentre/factsheets/fs310/en/index.html>) are related to particle exposure, and contact with PM will increase human morbidity and mortality. This particle related morbidity and mortality include lower respiratory infections, chronic

CLINICAL RELEVANCE

This research concerns the disruption in iron homeostasis that occurs in the pleura and lungs of patients treated with talc pleurodesis. The accumulation of this metal, the accompanying oxidative stress, and inflammatory events after exposure to talc are comparable to those with other forms of particulate matter. Pleurodesis can function as a model of particle related biological effect.

obstructive pulmonary disease, respiratory cancers, coronary heart disease, stroke, and other cerebrovascular diseases (1-3).

The production of reactive oxygen species (ROS) is fundamental to the biological effect of PM (4), but the specific mechanism of both the generation of oxidants and its relationship with the biological effect after exposure to particles is incompletely understood. One postulate to explain the biological effect after exposure to particles involves altered iron homeostasis in the host after exposure. Exposure to particles introduces a solid liquid interface into cells. Oxygen containing functional groups on the PM surface provide the capacity to complex cations, and as a result of its high affinity for oxygen donor ligands, iron is frequently preferred. Such functional groups can include alcohols, aldehydes, and carboxylates on incompletely combusted carbon (e.g., cigarette smoke, diesel exhaust, and ambient air pollution particles), and silanol groups on silica and silicates. Particles retained in the lung consistently demonstrate a capacity to disrupt iron homeostasis and accumulate host metal (5). Endpoints reflecting oxidative stress and a biological effect can be correlated with this accumulation of iron that follows *in vivo* exposure to particles (6).

Pathologic processes observed in lungs among individuals exposed to particles include both inflammation and fibrosis (4, 7). In patients with recurrent pleural effusions, the fibro inflammatory properties of mineral oxide particles are exploited therapeutically with the instillation of massive quantities (i.e., grams) of talc into the pleural space to provide sclerosis (8). This therapeutic use affords an opportunity to examine human tissue for evidence of a disruption in iron homeostasis after an extreme exposure to mineral oxide particles. We accordingly tested the postulates that (1) *in vitro* exposure to talc induces a disruption of iron homeostasis, oxidative stress, and a biological effect, and (2) talc pleurodesis in humans is also associated with altered iron homeostasis and an accumulation of metal.

MATERIALS AND METHODS

Iron Uptake in an Acellular Environment

Talc (Sclerosol, Bryan Corp., Woburn, MA) was agitated in either H₂O or 1,000 µM ferric ammonium citrate for 1 hour, centrifuged, and washed with H₂O (designated talc and talc Fe, respectively). Ionizable metal concentrations associated with talc and talc Fe were measured

(Received in original form May 27, 2011; accepted in final form August 5, 2011)

Disclaimer: This report was reviewed by the National Health and Environmental Effects Research Laboratory of the United States Environmental Protection Agency and was approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Correspondence and requests for reprints should be addressed to Andrew Ghio, Division of Environmental Public Health, Human Studies Facility, United States Environmental Protection Agency, Campus Box 7315, 104 Mason Farm Road, Chapel Hill, NC 27711. E-mail: ghio.and@epa.gov

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 46, Iss. 1, pp 80-86, Jan 2012

Originally Published in Press as DOI: 10.1165/rctmb.2011-0168OC on November 17, 2011
Internet address: www.atsjournals.org

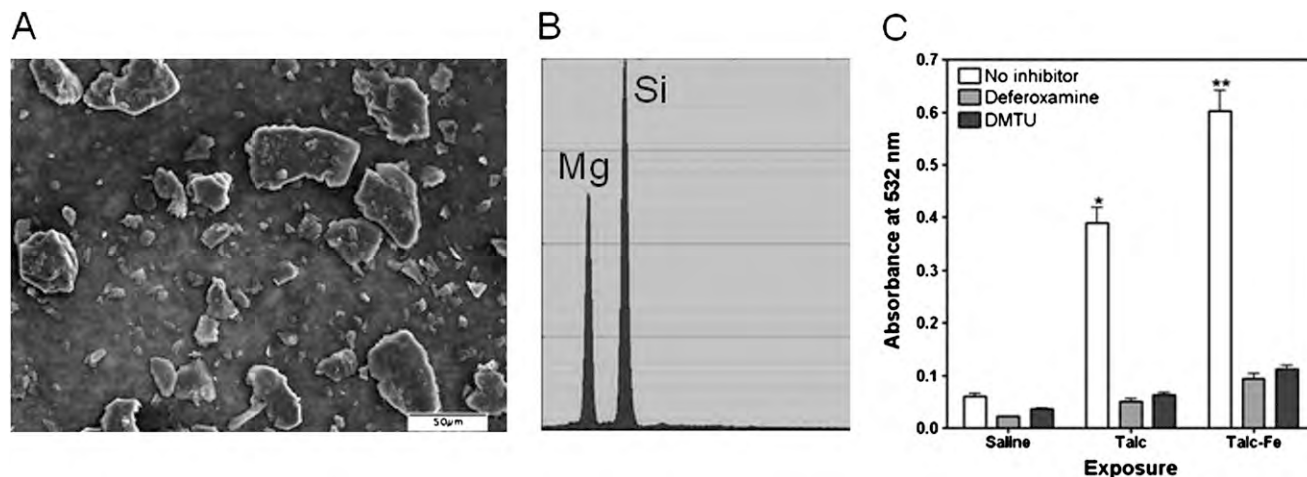


Figure 1. Particle size, composition, and capacity for generation of oxidants. (A) Talc was examined in a JEOL (Peabody, MA) scanning electron microscope at a screening magnification of approximately $\times 1,000$ and a diameter ranging between 10 and 50 μm . (B) Energy dispersive X ray spectroscopy confirmed two peaks consistent with magnesium and silicon. (C). Talc demonstrated a capacity to generate hydroxyl radical in an acellular environment. This generation of oxidants was increased with a further complexation of surface iron by the talc, and was inhibited by inclusion of the metal chelator deferoxamine or the hydroxyl radical scavenger dimethylthiourea (DMTU) during incubation. *Significantly increased relative to saline. **Significantly increased relative to both saline and talc.

using inductively coupled plasma optical emission spectroscopy (ICPOES; Model Optima 4300DV; Perkin Elmer, Norwalk, CT).

polymerase, with detection on an ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA).

Generation of Acellular Oxidants

The generation of acellular oxidants by talc and talc-Fe particles was measured with the thiobarbituric acid reactive products of deoxyribose (9).

Cell Culture

Mesothelial cells (MeT 5A; American Type Culture Collection, Manassas, VA) were cultured in complete growth medium. In addition, BEAS 2B cells on uncoated, plastic 12 well plates in keratinocyte growth medium (Lonza, Walkersville, MD) were used.

RT PCR

Mesothelial and BEAS 2B cells were exposed to either medium alone or 100 $\mu\text{g}/\text{ml}$ talc. Quantitative PCR was performed using Taqman

Cell Iron Homeostasis

Mesothelial and BEAS 2B cells were exposed for 4 hours to 200 μM ferric ammonium citrate (FAC), 100 $\mu\text{g}/\text{ml}$ talc, and both FAC and talc. Cells were then scraped into 1.0 ml 3 N HCl/10% trichloroacetic acid. After hydrolysis, concentrations of iron and zinc were determined using ICPOES. Cell incubations were repeated for 24 hours, scraped into 0.5 ml PBS, and disrupted, and ferritin concentrations in the lysates were measured using an enzyme immunoassay (Microgenics Corp., Concord, CA).

Generation of Cellular Oxidants

Mesothelial and BEAS 2B cells were grown to confluence in 96 well, white walled, tissue culture treated plates (CoStar, Lowell, MA). We loaded 2',7'-dichlorodihydrofluorescein (DCF; 20 μM) diacetate (Sigma

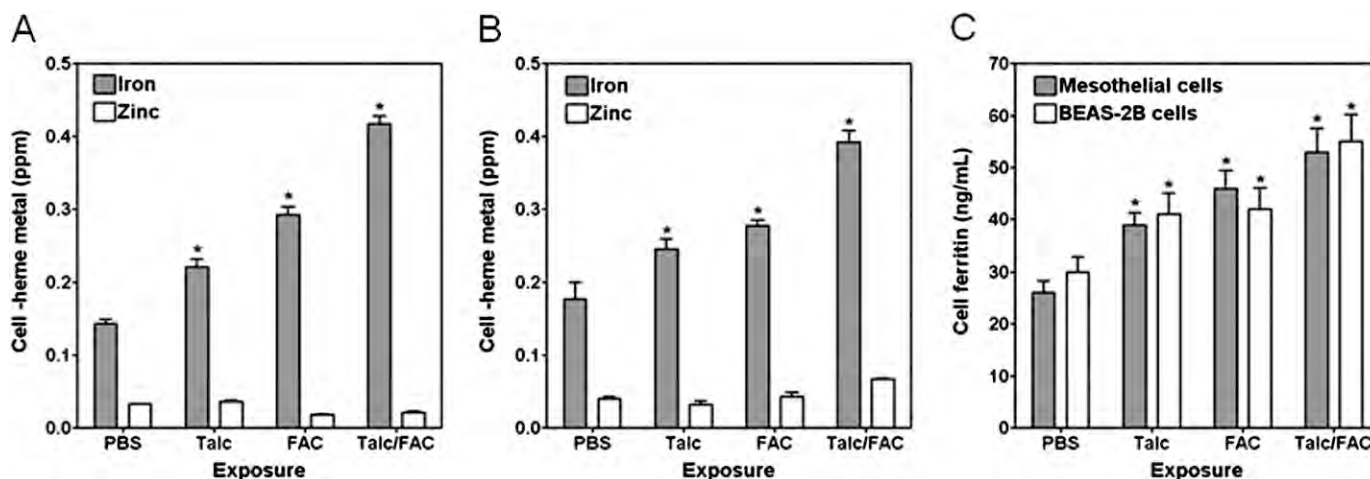


Figure 2. Cellular concentrations of nonheme metal and ferritin after *in vitro* exposure to talc. Mesothelial (A) and BEAS 2B cells (B) both increased cell nonheme iron after exposure to 100 $\mu\text{g}/\text{ml}$ talc and 200 μM iron. The co incubation of cells with both talc and iron further elevated nonheme iron. Cell zinc concentration showed no change after exposures to talc and iron. (C) Corresponding to concentrations of nonheme iron, concentrations of cell ferritin increased in both cell types after exposures to talc and iron. *Significantly increased, relative to PBS. FAC, ferric ammonium citrate.

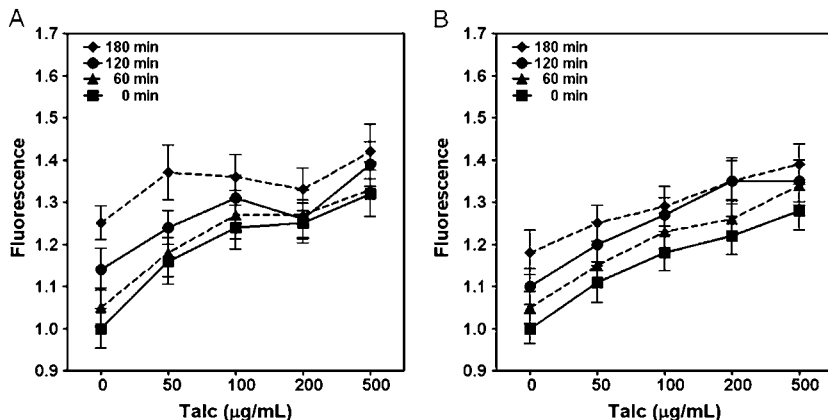


Figure 3. Cellular generation of oxidants after *in vitro* exposure to talc. Using 2'7' dichlorodihydrofluorescein (DCF) diacetate fluorescence, mesothelial cells (A) and BEAS 2B cells (B) demonstrated a time dependent and concentration dependent generation of oxidants.

Chemical Co., St. Louis, MO), baseline readings were taken on a Perkin Elmer HTS 7000 fluorimeter using 485 nm excitation/535 nm emission filters, and PBS with and without talc was added. Fluorescence was measured at 0, 60, 120, and 180 minutes after addition.

Cellular Release of IL 8 and IL 6

Cells were exposed to either medium or 100 $\mu\text{g/ml}$ talc in medium for 24 hours. Concentrations of IL 8 and IL 6 in the cell medium were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN).

Histochemistry and Immunohistochemistry

Our protocol was approved by the Institutional Review Board of the Duke University Health System. Staining was performed on six surgical specimens collected from patients who (1) had manifested talc pleurodesis 2–15 months previously (mean \pm SD, 7 ± 6 months), followed by (2) an extrapleural pneumonectomy. Sections were stained for iron, using Perl's Prussian blue (Sigma). Immunohistochemistry for ferritin was performed using an human anti ferritin antibody (Dako, Carpinteria, CA) at a dilution of 1:100 (10) and antibodies to divalent metal transport 1 (DMT1) and ferroportin 1 (FPN1) at a dilution of 1:200 (11, 12). Control lung tissue was obtained from (1) patients who had undergone a pneumonectomy for lung cancer, and (2) individuals diagnosed with idiopathic pulmonary fibrosis (IPF) and undergoing an autopsy.

Statistical Analysis

Data are expressed as mean values \pm standard errors, unless specified otherwise. Differences between multiple groups were compared using ANOVA.

RESULTS

Scanning electron microscopy of the talc showed significant variability in PM size, with diameters of individual particles ranging from less than 10 μm to greater than 50 μm (Figure 1A). However, the majority of particles had diameters between 10 and 50 μm . No fibers were evident in the talc sample. Energy dispersive X ray spectroscopy revealed two peaks consistent with magnesium and silicon (Figure 1B). This finding is in agreement with the ideal molecular formula of talc ($\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$).

Comparable to numerous silicates (13), talc demonstrated a capacity for iron uptake in an acellular environment. Concentrations of ionizable iron were measured at 0.46 ± 0.11 ppm and 6.48 ± 1.08 ppm, respectively, for talc and talc Fe, whereas concentrations of ionizable zinc were measured at 0.03 ± 0.01 and 0.01 ± 0.00 ppm, respectively. The capacity of particles to support the *in vitro* generation of oxidants in an acellular environment was significantly affected by the concentration of associated iron, with talc Fe producing a significantly greater signal for malondialdehyde relative to talc (Figure 1C). This generation of oxidants was inhibited by the inclusion of either 1,000 μM deferoxamine, a metal chelator, or 1,000 μM dimethylthiourea, a hydroxyl radical scavenger (Figure 1C).

The *in vitro* exposure of mesothelial cells to talc for 4 hours resulted in an accumulation of nonheme iron. Cell zinc concentrations did not change (Figure 2A). The exposure to FAC alone confirmed the ability of mesothelial cells to import iron (Figure 2A). Co incubation with both talc and FAC was associated with a greater accumulation of iron relative to FAC

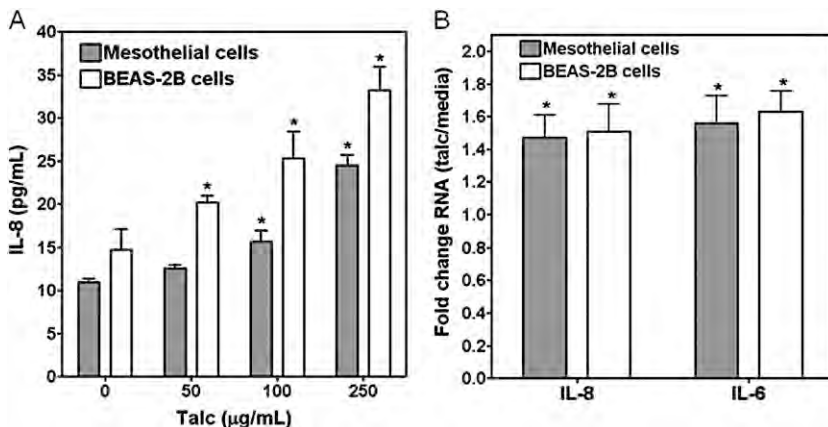


Figure 4. Release of IL 8 and changes in RNA for IL 8 and IL 6 after *in vitro* exposure to talc. (A) Concentrations of IL 8 were increased after exposure to 100 $\mu\text{g/ml}$ talc in both cell types. (B) Changes in RNA for IL 8 and IL 6 were increased 4 hours after exposure to 100 $\mu\text{g/ml}$ talc. * significantly increased relative to PBS (A) and media (B).

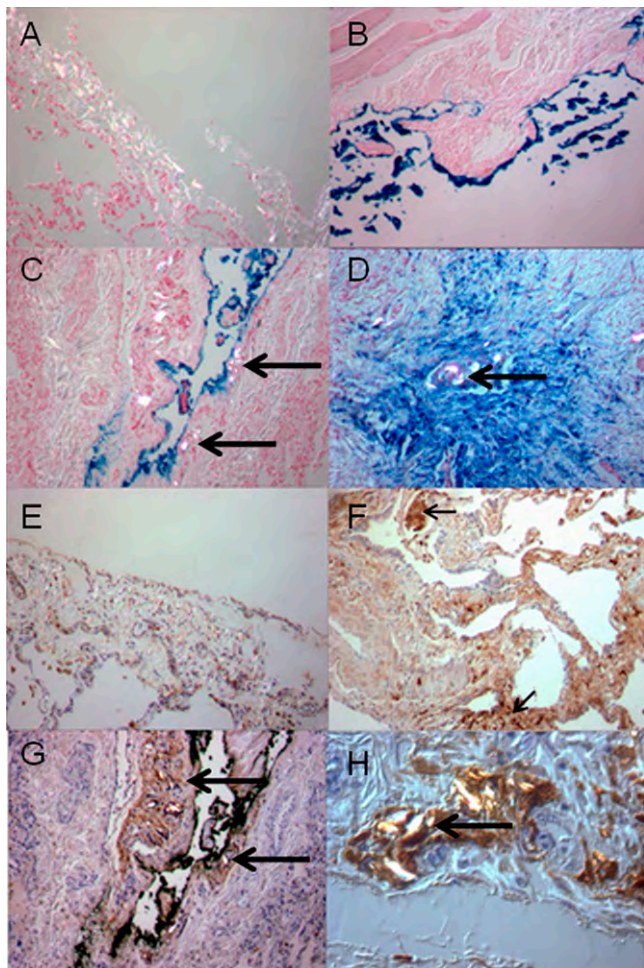


Figure 5. Stains for iron and ferritin in resected lung tissues. (A) Pleura from control lung tissue demonstrated no staining for iron by the pleura. Those tissue samples acquired after pleurodesis all showed positive iron staining. This staining included the parietal pleura (B) and visceral pleura (C). With translocation of the talc to both the subpleural region (C; arrows designate talc particles) and the parenchyma (D; arrow designates talc particle) of the lung, positive iron staining was evident in cells adjacent to the particle (D). (E) A small amount of uptake for the ferritin antibody by the pleura occurred in samples resected from patients treated with pneumonectomy. Specifically, this staining localized to the single cell layer of mesothelial cells and the loose fibrous tissue immediately beneath it. (F) Some expression of ferritin was also evident in chronic inflammatory cells resident in the lungs of patients with idiopathic pulmonary fibrosis (IPF; arrows designate areas of chronic inflammation distorting the alveolar and septal regions). (G) However, in patients with pleurodesis, talc was evident in the subpleural region, and uptake of the ferritin antibody by the pleura was very strong (arrows designate particles). (H) Talc was also detected in the lung parenchyma, and ferritin staining was similarly increased in cells adjacent to the particle (arrow designates particle). The expression of ferritin in the lungs of patients with pleurodesis was focally distributed to sites of talc retention. Magnification, approximately $\times 100$.

exposure alone (Figure 2A). BEAS 2B cells exposed to talc and FAC revealed similar increases in cell nonheme metal. Again, co incubations revealed an increased cell iron import relative to either talc or FAC alone (Figure 2B). These results demonstrate that the capacity of talc to complex iron in an acellular environment is retained during *in vitro* mesothelial and airway epithelial cell exposures. In addition, these data suggest that talc

could deplete intracellular sources of iron, and resulting in the importation of greater quantities of metal. Comparable to cell nonheme iron concentration, an increase in cell ferritin occurred after exposures of mesothelial and airway epithelial cells to talc, and this increase was even greater after exposures to both FAC and talc (Figure 2C). RNA for ferritin, DMT1 (a major iron importer), and FPN1 (a major iron exporter) did not significantly change 4 hours after exposure of mesothelial cells to 100 $\mu\text{g/ml}$ talc (Table E1 in the online supplement).

A fluorescence method using DCF diacetate demonstrated an increased generation of ROS by both mesothelial and BEAS 2B cells after exposure to talc (Figures 3A and 3B). This cellular generation of oxidants after exposure to particles was both time dependent and concentration dependent, supporting a capacity of cells to produce ROS after exposure to talc. RNA for heme oxygenase and cyclooxygenase, which are potential intracellular sources of oxidative stress, showed conflicting responses, with heme oxygenase significantly increasing in both mesothelial and airway epithelial cells 4 hours after exposure to 100 $\mu\text{g/ml}$ talc, and the concentration of cyclooxygenase not changing (Table E1). Finally, mesothelial and BEAS 2B cells both increased the release of IL 8 after exposure to 100 $\mu\text{g/ml}$ talc (Figure 4A). IL 6 demonstrated a trend toward increasing after exposure to talc, but significant differences were not evident. RNA for IL 8 and IL 6 similarly increased after a 4 hour exposure to talc in both mesothelial cells and BEAS 2B cells (Figure 4B). This increased release of proinflammatory mediators reflects a relevant *in vitro* biological response in both cell types after talc exposure.

All patients with pleurodesis were male. Their mean age (\pm SD) was 56 ± 18 years. All but one manifested malignant mesothelioma (the exception underwent surgical intervention for repeated pneumothoraces). Tissue specimens, used as control samples, were collected at the time of pneumonectomy for lung cancer ($n = 6$) and at autopsy of patients with IPF ($n = 6$). These individuals manifested no pleurodesis. The specimens obtained during pneumonectomy reflected tissue uninvolved by fibrosis, whereas specimens from patients diagnosed with IPF included fibrotic specimens unrelated to mineral oxide exposure. Control tissue collected during pneumonectomy and autopsy were from men with mean ages (\pm SD) of 70 ± 15 years and 68 ± 9 years, respectively. Control lungs demonstrated no staining for iron in the pleura or parenchyma (Figure 5A). Significant iron staining occurred in all specimens collected after pleurodesis. Iron was localized to both the parietal (Figure 5B) and visceral (Figure 5C) pleura. Some of the talc was observed to be subpleural (Figure 5C), and particles were also evident in the parenchyma of the lungs (Figure 5D). When particles moved subpleurally and into the parenchyma, they retained their capacity to accumulate the metal (Figure 5D). Some uptake of the ferritin antibody by pleura occurred in the control specimens collected from patients with lung cancer (Figure 5E) and IPF, but this uptake was minimal. Among patients with IPF, uptake for the ferritin body corresponded to areas of inflammation (Figure 5F). After exposure to talc, the staining for ferritin was so intense that the visceral pleura appeared black (Figure 5G). After transport to the lung parenchyma, talc continued to demonstrate an association with an increased expression of ferritin protein (Figure 5H). The expression of this storage protein was focally distributed to the talc, with increased staining immediately adjacent to the particle.

Little staining for DMT1 was evident in the pleura of lungs resected from control patients, and this staining was limited to respiratory epithelia (Figures 6A and 6B). The expression of this major iron importer in the pleura increased enormously after pleurodesis with talc (Figure 6C). Uptake for the iron

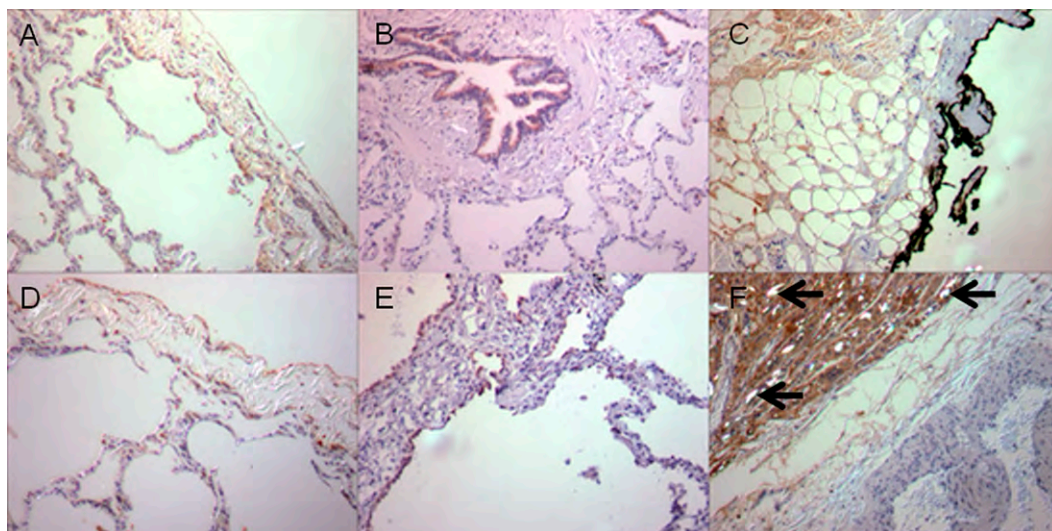


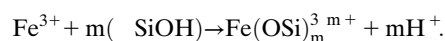
Figure 6. Divalent metal transport 1 (DMT1) and ferroportin 1 (FPN1) staining in resected lung tissue. Some binding of the antibody to both DMT1 and FPN1 was evident in lung tissue resected from patients unexposed to talc (A and D, respectively). Similarly, the uptake of antibodies for DMT1 and FPN1 in autopsy samples from patients with IPF was low (B and E, respectively). However, the expression of the iron importer DMT1 and iron exporter FPN1 both increased significantly among patients treated with pleurodesis (C and F, respectively). Comparable to the expression of ferritin, increased uptake for the antibodies to DMT1 and FPN1 occurred after translocation of the particle to the lung parenchyma (F; arrows designate particles). Magnification, approximately $\times 100$.

exporter FPN1 was negligible among lungs resected from control patients (Figures 6D and 6E). Among those patients with talc pleurodesis, the expression of FPN1 was greatly increased at the pleura and in the lung cells immediately adjacent to translocated particles (Figure 6F). Finally, trichrome staining for collagen demonstrated minuscule, subpleural staining in the control specimens taken during pneumonectomy for lung cancer (Figure 7A). In contrast, sheets of collagen were verified by trichrome staining in lung tissue resected after talc pleurodesis (Figure 7B). In the lungs of patients with pleurodesis, the talc appeared at the periphery of the fibrosis (Figure 7B).

DISCUSSION

The surfaces of silica and silicate particles, including talc, contain some concentration of silanol groups (Si OH). Si^{4+} has a high electron affinity, and the Si O bond consequently has a significant ionic character and an acidic dissociation constant favoring dissociation at physiologic pH (14). The dissociation of silanol groups contributes to a net negative charge on the particle surface, which generates a capacity for the adsorption and exchange of cations (15). The open network of negatively charged silanol groups on a silica and silicate surface presents spaces large enough to accommodate adsorbed metal cations. As a result of its electropositivity,

Fe^{3+} has a high affinity for oxygen donor ligands (16, 17), and reacts with the silanol group to form a silicato iron coordination complex (18):



The dose dependent adsorption of inorganic iron was demonstrated for surface silanol groups on crystalline silicates, with critical stability constants up to $1 \times 10^{17.15}$ (critical stability constant = 17.15) (19, 20). Our investigation confirmed the capacity of talc to complex iron from an acellular source comparable to other silica and silicate particles (13). The *in vitro* exposure to talc similarly affected an accumulation of nonheme iron in both mesothelial and airway epithelial cells. Although the source of this accumulated iron was not identified, the talc surface may complex host metal originally associated with ATP, ADP, GTP, citrate, DNA, free amino acids (21), and mitochondrial sources. However, the cell will recognize this loss of requisite iron, and subsequently increases metal import. After integration of the particle's capacity for complexation into the cell's requirement for iron, increased metal uptake and storage would occur. Reflecting these processes, concentrations of ferritin increased after *in vitro* exposure to talc. As a result of posttranscriptional control acting as a major contributor to expression (22–24), the

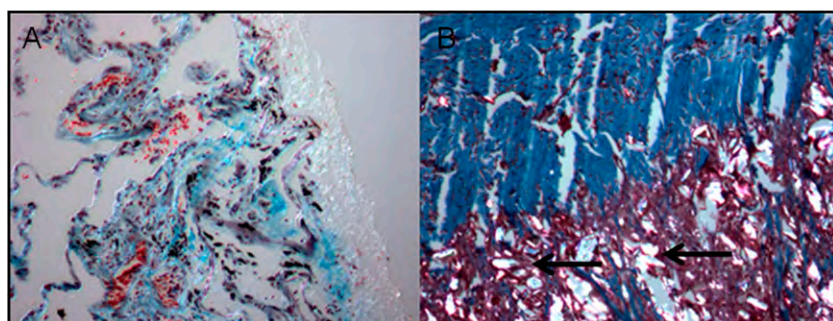


Figure 7. Masson's trichrome stain for collagen in resected lung tissue. (A) Control lungs demonstrated little trichrome staining (blue). (B) In contrast, tissue collected from patients with pleurodesis showed dense sheets of collagen. Talc was evident at the periphery of this collagen deposition, comparable to a silica nodule (arrows designate particles). Magnification, approximately $\times 100$.

RNA for ferritin, DMT1, and FPN1 did not change in mesothelial and BEAS 2B cells.

Comparable to *in vitro* accumulation of iron after exposure to talc, significant metal accumulation occurred *in vivo* after pleurodesis. Perl's Prussian blue staining demonstrated metal in close proximity to retained talc particles in both the mesothelial cells of the pleura and the macrophages and airway epithelial cells of the parenchyma. Because the iron originally associated with the talc approximated normal cell and tissue concentrations, the accumulation of metal after pleurodesis had to originate from host sources of iron. Immunohistochemistry for ferritin showed elevations in expression by mesothelial cells at the pleura and in parenchymal cells after the introduction of talc. The stain for ferritin suggested a very focal response, with cells either directly contacting or immediately adjacent to the particle demonstrating uptake of the antibody to this storage protein. Similarly, the expression of the iron importer DMT1 and the iron exporter FPN1 both increased on staining in those patients treated with talc pleurodesis. In control tissue from patients with lung cancer and IPF, no positive staining for iron was evident. The uptake of the antibody to ferritin, DMT1, and FPN1 in these same tissues was minimal, but was evident in mesothelial cells of the visceral pleura, airway epithelial cells, and alveolar macrophages. In addition, ferritin was evident in the chronic inflammatory cells of autopsy samples from patients with IPF. The evidence for disrupted iron homeostasis in IPF was not compelling and this pathway is not proposed as a mechanism of biological effect in all fibrotic injuries.

Elevations in tissue concentrations of ferritin, DMT1, and FPN1 in tissues collected after pleurodesis challenge current understanding, because the controls of expression can be diametrically opposite to each other (22). The expression of these three proteins involves the same posttranscriptional mechanism, using the iron responsive element (IRE). For ferritin and FPN1, a specific sequence at the 5' untranslated end of ferritin mRNA (i.e., the IRE) binds a cubane iron sulfur cluster, referred to as the iron regulatory protein (IRP), when the IRP exists in the apoprotein form. Elevated concentrations of available iron react with IRP to alter its conformation, decrease affinity of the protein to the mRNA, and displace it from the mRNA, allowing translation to proceed. In contrast, DMT1 mRNA contains an IRE at the 3' untranslated region that allows increased synthesis with iron depletion. The elevation in expression of all three of these proteins after pleurodesis supports an iron depletion that occurs after the complexation of essential cell metal by the surface of the endocytosed talc particle. This initiates an increase in intracellular iron as the cell elevates concentrations to meet the demands of a new equilibrium imposed by the talc. Subsequently, all three proteins can be elevated, but such expression may involve temporal and regional variability.

The disruption of cell iron homeostasis is frequently associated with oxidative stress (25, 26). In a similar manner, *in vitro* exposure to talc increased the generation of oxidants, measured as DCF diacetate fluorescence, by mesothelial and airway epithelial cells. The acellular assay for hydroxyl radical production suggests that surface functional groups on the talc complex a source of available iron in the cell, which then redox cycles, generating oxidant. An alternative proposal could involve talc sequestering cell iron and the host response involving superoxide generation as a ferrireductant to resecure the requisite metal. *In vitro* changes in heme oxygenase RNA after cellular exposures to talc also support oxidative stress.

The biological effect of talc was evaluated using indices of the fibro inflammatory response. The *in vitro* cellular response to

talc included the increased release of IL 8 and an elevation of RNA for both IL 8 and IL 6, comparable to those for many particles (27). Among patients treated with talc pleurodesis, the deposition of collagen was obvious on trichrome staining, again analogous to numerous particle exposures (6). The indices of both inflammation and fibrosis after exposure to mineral oxide particles were demonstrated to correlate with changes in iron homeostasis. The release of inflammatory mediators can directly correspond to the concentration of metal complexed to a particle surface (28). Regarding the relationship between iron and fibrotic injury, exposure to metal chelators such as bleomycin can increase the activity of prolyl hydroxylase, resulting in a deposition of collagen (29). *In vitro* and *in vivo* exposures both confirm that biological effects after talc include those proinflammatory and fibrotic events previously associated with mineral oxide particles.

We conclude that exposure to talc disrupts iron homeostasis in mesothelial and airway epithelial cells, and is associated with both oxidative stress and a biological effect, comparable to those of other particles. The resultant accumulation of iron and alterations in iron related proteins are evident among patients with pleurodesis. Sclerosis after exposure to talc can be regarded as a model of therapeutic benefit after a massive and focal exposure to a mineral oxide particle.

Author Disclosure: A.J.G., J.M.S., L.A.D., J.H.R., J.L.T., and E.N.P. do not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. V.L.R. has testified in cases involving talc manufacturers in asbestos litigation.

References

1. Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, et al. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation* 2010;121:2331-2378.
2. Pelucchi C, Negri E, Gallus S, Boffetta P, Tramacere I, La Vecchia C. Long term particulate matter exposure and mortality: a review of European epidemiological studies. *BMC Public Health* 2009;9:453.
3. Zhang JJ, Smith KR. Household air pollution from coal and biomass fuels in China: measurements, health impacts, and interventions. *Environ Health Perspect* 2007;115:848-855.
4. Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med* 1998;157:1666-1680.
5. Ghio AJ, Churg A, Roggli VL. Ferruginous bodies: implications in the mechanism of fiber and particle toxicity. *Toxicol Pathol* 2004;32:643-649.
6. Ghio AJ, Jaskot RH, Hatch GE. Lung injury after silica instillation is associated with an accumulation of iron in rats. *Am J Physiol* 1994; 267:L686-L692.
7. Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsey DM, Pretty JR, McLaurin JL, et al. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation: potential role of free radicals. *Am J Respir Crit Care Med* 1995;152:1003-1009.
8. Rodriguez Panadero F, Antony VB. Pleurodesis: state of the art. *Eur Respir J* 1997;10:1648-1654.
9. Ghio AJ, Kennedy TP, Stonehuerner JG, Crumbliss AL, Hoidal JR. DNA strand breaks following *in vitro* exposure to asbestos increase with surface complexed (Fe³⁺). *Arch Biochem Biophys* 1994;311:13-18.
10. Wang X, Ghio AJ, Yang F, Dolan KG, Garrick MD, Piantadosi CA. Iron uptake and NRAMP2/DMT1/DCT1 in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L987-L995.
11. Ghio AJ, Wang X, Silbajoris R, Garrick MD, Piantadosi CA, Yang F. DMT1 expression is increased in the lungs of hypotransferrinemic mice. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L938-L944.
12. Yang F, Liu XB, Quinones M, Melby PC, Ghio A, Haile DJ. Regulation of reticuloendothelial iron transporter MTP1 (SLC11A3) by inflammation. *J Biol Chem* 2002;277:39786-39791.
13. Ghio AJ, Kennedy TP, Whorton AR, Crumbliss AL, Hatch GE, Hoidal JR. Role of surface complexed iron in oxidant generation

- and lung inflammation induced by silicates. *Am J Physiol* 1992;263: L511 L518.
14. Iler RK. The chemistry of silica. New York: John Wiley and Sons; 1979.
 15. Grim RE. Clay mineralogy. New York: McGraw Hill Book Co.; 1968.
 16. Kragten J. Atlas of metal ligand equilibria in aqueous solution. New York City: Halstead Press; 1978.
 17. Crumbliss ALGJ. A comparison of some aspects of the coordination chemistry of aluminum (III) and iron (III). *Comm Inorg Chem* 1988;8: 1 26.
 18. Dugger DLSJ, Irby BN, McConnell BL, Cummings WW, Mattman RW. The exchange of twenty metal ions with the weakly acidic silanol group of silica gel. *J Phys Chem* 1964;68:757 760.
 19. Fordham AW. Sorption and precipitation of iron on kaolinite: factors involved in sorption equilibria. *Aust J Soil Res* 1969;7:185 197.
 20. Herrera HRP. Reaction of montmorillonite with iron (III). *Proc Soil Sci Soc Am* 1970;34:740 745.
 21. Breuer W, Epsztejn S, Cabantchik ZI. Iron acquired from transferrin by K562 cells is delivered into a cytoplasmic pool of chelatable iron (II). *J Biol Chem* 1995;270:24209 24215.
 22. Muckenthaler MU, Galy B, Hentze MW. Systemic iron homeostasis and the iron responsive element/iron regulatory protein (IRE/IRP) regulatory network. *Annu Rev Nutr* 2008;28:197 213.
 23. Nunez MT. Regulatory mechanisms of intestinal iron absorption: uncovering of a fast response mechanism based on DMT1 and ferroportin endocytosis. *Biofactors* 2010;36:88 97.
 24. Thomson AM, Rogers JT, Leedman PJ. Iron regulatory proteins, iron responsive elements and ferritin mRNA translation. *Int J Biochem Cell Biol* 1999;31:1139 1152.
 25. Caporossi D, Ciafre SA, Pittaluga M, Savini I, Farace MG. Cellular responses to H₂O₂ and bleomycin induced oxidative stress in I6C5 rat myoblasts. *Free Radic Biol Med* 2003;35:1355 1364.
 26. Ghio AJ, Hilborn ED, Stonehuerner JG, Dailey LA, Carter JD, Richards JH, Crissman KM, Foronjy RF, Uyeminami DL, Pinkerton KE. Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect. *Am J Respir Crit Care Med* 2008;178:1130 1138.
 27. Seagrave J. Mechanisms and implications of air pollution particle associations with chemokines. *Toxicol Appl Pharmacol* 2008;232:469 477.
 28. Pritchard R, Ghio AJ, Lehmann J, Park P, Gilmour MI, Winsett DW, Dreher KL, Costa DL. Oxidant generation and lung injury after exposure to particulate air pollutants are associated with concentration of complexed iron. *Inhal Toxicol* 1996;8:457 477.
 29. Giri SN, Misra HP, Chandler DB, Chen ZL. Increases in lung prolyl hydroxylase and superoxide dismutase activities during bleomycin induced lung fibrosis in hamsters. *Exp Mol Pathol* 1983;39:317 326.

Exhibit 92

Cytotoxicity and Apoptosis Induction by Nanoscale Talc Particles from Two Different Geographical Regions in Human Lung Epithelial Cells

Mohd Javed Akhtar,^{1,2} Maqsood Ahamed,³ M. A. Majeed Khan,³ Salman A. Alrokayan,³ Iqbal Ahmad,² Sudhir Kumar¹

¹Fibre Toxicology Division, Indian Institute of Toxicology Research, Lucknow 226001, India

²Department of Zoology, University of Lucknow, Lucknow 226007, India

³King Abdullah Institute for Nanotechnology, King Saud University, Riyadh 11451, Saudi Arabia

Received 25 October 2012; revised 16 January 2012; accepted 21 January 2012

ABSTRACT: We have characterized the physicochemical properties of nanotalc particles from two different geographical regions and examined their toxicity mechanisms in human lung epithelial (A549) cells. Indigenous nanotalc (IN) of Indian origin and commercial nanotalc (CN) of American origin were used in this study. Physicochemical properties of nanotalc particles were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), Brunauer-Emmet-Teller (BET), and dynamic light scattering (DLS). Results showed that both IN and CN particles significantly induce cytotoxicity and alteration in cell cycle phases. Both IN and CN particles were found to induce oxidative stress indicated by induction of reactive oxygen species (ROS), lipid peroxidation, and depletion of antioxidant levels. DNA fragmentation and caspase-3 enzyme activation due to IN and CN particles exposure were also observed. We further showed that after iron chelation, IN and CN particles produce significantly less cytotoxicity, oxidative stress, and genotoxicity to A549 cells as compared with nonchelated particles. In conclusion, this study demonstrated that redox active iron plays significant role in the toxicity of IN and CN particles, which may be mediated through ROS generation and oxidative stress. © 2012 Wiley Periodicals, Inc. *Environ Toxicol* 29: 394–406, 2014.

Keywords: nanotalc particles; physicochemical characterization; iron chelation; toxicity; apoptosis

INTRODUCTION

Talc is a mineral compound $[\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2]$ with unique attributes and significant commercial importance.

Correspondence to: M. Ahamed; e mail: maqsood@gmail.com or mahamed@ksu.edu.sa

Contract grant sponsor: King Abdulaziz City for Science and Technology (KACST) under the National Plan for Science and Technology (NPST).

Contract grant number: 10 NAN1201 02

Contract grant sponsor: University Grants Commission (UGC), New Delhi, India (to M.J.A.)

Published online 13 February 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/tox.21766

Talc is widely used due to its intrinsic properties such as high thermal stability, low electrical conductivity, good absorption and adsorption properties, and high crystallinity (Pérez-Maqueda et al., 2005; Nkoubou et al., 2008). Talc is utilized in various applications including paper, paint, cosmetic, plastic, ceramic, pesticide, and pharmaceuticals (Carretero, 2002; Bizi et al., 2003; Petit et al., 2004). Hence, occupational and consumer exposures to talc particles are wide and complex (Jaynes and Zartman, 2005). It has been reported that talc mine workers show higher rates of lung cancer and other respiratory diseases (National Toxicology Program, 1993). Epidemiologic evidence also suggests a possible association between genital use of talcum powder and risk of ovarian cancer (Wild,

2006; Buz'Zard and Lau, 2007; Gates et al., 2008; Langseth et al., 2008). Talc also appears to induce reactive oxygen species (ROS) generation, oxidative stress, and inflammation (Harlow and Hartge, 1995; Buz'Zard and Lau, 2007).

Due to enhanced intrinsic properties, nanoscale talc particles are extensively utilized in many commercial and industrial products (Akhtar et al., 2008; Balamurugan and Maiti, 2010; Sakthivel and Pitchumani, 2011). Despite the wide-spread applications, there is a serious lack of information concerning the mechanisms of toxicity of nanotalc particles. Previously, we have observed that human cells exposed to nanotalc particles induce oxidative stress-mediated cytotoxicity (Akhtar et al., 2010a). However, physicochemical characterization of nanotalc particles and their association with the toxicological response in human cells is still remains unclear.

There are numerous reports suggesting that ROS is an important mediator of the toxicity of minerals such as asbestos and silica (Aung et al., 2007; Akhtar et al., 2010b). It has been known for years that the surface iron (II) or leachable iron (II) on mineral surfaces reduces molecular oxygen to superoxide anion, which is then dismutated to hydrogen peroxide (Shukla et al., 2003). In the presence of asbestos or silica, hydrogen peroxide and superoxide react via a Fenton reaction and/or Haber Weiss reaction driven by iron to form the potent hydroxyl radical in vitro leading to cellular oxidative damage (Persson et al., 2003).

The aim of this work was to characterize the physicochemical properties of nanotalc particles and to determine the role of iron in the toxicity mechanisms of nanotalc particles in human lung epithelial (A549) cells. We utilized two types of nanotalc particles from different geographical origins; indigenous nanotalc (IN) of Indian origin and commercial nanotalc (CN) of American origin. Cytotoxicity of IN and CN particles was examined by MTT and LDH assays. Oxidative stress response of IN and CN particles was assessed by measuring reactive oxygen species (ROS), lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Apoptotic response of IN and CN particles was evaluated by cell cycle analysis, DNA fragmentation, and caspase-3 enzyme activity. To explore the role of iron in the toxicity of IN and CN particles, we utilized deferoxamine mesylate (DFOM), a well-known iron chelator. The physicochemical properties of IN and CN particles were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), Brunauer-Emmet-Teller (BET), and dynamic light scattering (DLS). The A549 cells, derived from human lung carcinoma, have been widely utilized in toxicological studies (Zhang et al., 2010; Akhtar et al., 2010a,b; Ahamed et al., 2011a,b,c).

MATERIALS AND METHODS

Nanotalc Particles and Reagents

We have utilized the nanotalc particles from two different geographical regions. Indigenous nanotalc (IN) particles were collected from Rajasthan, India, as reported in our previous publication (Akhtar et al., 2010a). American origin commercial nanotalc (CN) particles (size 70 12 nm) were purchased from M.K. Impex (Mississauga, Canada).

Fetal bovine serum (FBS), penicillin-streptomycin, DMEM/F-12 medium, and HBSS were purchased from Invitrogen Co. (Carlsbad, CA). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide], 2,7-dichlorofluorescein diacetate (DCFH-DA), deferoxamine mesylate (DFOM), glutathione (GSH), thiobarbituric acid (TBA), propidium iodide (PI), RNase A, diethylenetriaminepentaacetic acid (DETAPAC), *N*-acetyl-asp-glu-val-asp-7-amido-4-trifluoromethylcoumarin (Ac-DEVD-AFC), 7-amido-4-trifluoromethylcoumarin (AFC) standard, Bradford reagent, and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO). Apoptotic DNA Ladder Kit was bought from Roche. All other chemicals used were of the highest purity available from commercial sources.

Characterization of Nanotalc Particles

Crystalline nature of both IN and CN particles were examined by taking X-ray diffraction (XRD) pattern at room temperature with the help of PANalytical X'Pert X-ray diffractometer equipped with a Ni filtered using Cu-K α (λ = 1.54056 Å) radiations as X-ray source. Morphology and size of IN and CN particles were examined by field emission transmission electron microscopy (FETEM) (JEM-2100F, JEOL Inc., Tokyo, Japan) at an accelerating voltage of 200 kV. To check the purity of IN and CN particles, an energy dispersive X-ray spectroscopy (EDS) analysis was performed. Brunauer-Emmet-Teller (BET) surface area measurement of IN and CN particles was determined by multipoint nitrogen adsorption at 77 K using a Beckman Coulter SA3100 device.

Dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) for the characterization of hydrodynamic size and zeta potential (ζ) of IN and CN particles in distilled water and cell culture media were performed on a Malvern Instruments Zetasizer Nano-ZS instrument as described by Murdock et al. (2008).

Treatment of Nanotalc Particles with Deferoxamine Mesylate

We treated both IN and CN particles with DFOM for iron chelation. In brief, IN and CN particles were incubated with 10 mM DFOM at a concentration of 1000 μ g/mL for

20 h as described by Aung et al. (2007). Then particles were washed three times with cell culture medium by centrifuging at 4000 rpm for 10 min followed by resuspension.

Cell Culture and Exposure to Nanotalc Particles

Human lung epithelial (A549) cells were obtained from National Centre for Science (NCCS), Pune, India. Cells were used between passages 10 and 20. Cells were cultured in DMEM/F-12 medium supplemented with 10% FBS and 100 U/mL penicillin-streptomycin at 5% CO₂ and 37°C. At 85% confluence, cells were harvested using 0.25% trypsin and were subcultured into 75 cm² flasks, 6-well plates, or 96-well plates according to selection of experiments. Cells were allowed to attach the surface for 24 h before treatment. IN and CN particles were suspended in cell culture medium and diluted to a appropriate concentration (200 µg/mL). Suspension of nanotalc particles were then sonicated using a sonicator bath at room temperature for 10 min at 40 W to avoid particles agglomeration before administration to the cells. The selection of the 200 µg/mL concentration of nanotalc particles was based on our previous publication (Akhtar et al., 2010a). All the data presented in this study was that of 48 h exposure. Cells not exposed to nanotalc particles served as controls in each experiment.

Cell Viability Assay

Viability of A549 cells after exposure to nanotalc particles was assessed by MTT assay as described by Mossman (1983). The MTT assay assesses the mitochondrial function by measuring ability of viable cells to reduce MTT into blue formazan product. In brief, 10,000 cells per well were seeded in 96-well plate and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. After the exposure completed, the medium was removed from each well to avoid interference of particles and replaced with new medium containing MTT solution in an amount equal to 10% of culture volume, and incubated for 3 h at 37°C until a purple colored formazan product developed. The resulting formazan product was dissolved in acidified isopropanol. Further, the 96-well plate was centrifuged at 2500 rpm for 5 min to settle down the remaining particles present in the solution. Then, a 100 µL supernatant was transferred to other fresh wells of 96-well plate and absorbance was measured at 570 by using a microplate reader (FLUOstar-Omega).

Lactate Dehydrogenase Leakage Assay

Lactate dehydrogenase (LDH) is an enzyme widely present in cytosol that converts lactate to pyruvate. When plasma

membrane integrity is disrupted, LDH leaks into culture media and its extracellular level is elevated. LDH assay was carried out with the method described earlier (Wroblewski and LaDue, 1955; Welder et al., 1991). In brief, 10,000 cells per well were seeded in 96-well plate and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. After the exposure completed, the 96-well plate was centrifuged at 2500 rpm for 10 min to get the cell culture media. Then, a 100 µL of culture media transferred to new fresh tube containing 100 µL of sodium pyruvate (2.5 mg/mL phosphate buffer) and 100 µL of reduced nicotinamide adenine dinucleotide (NADH) (2.5 mg/mL phosphate buffer) in a total volume of 3.0 mL (0.1 M potassium phosphate buffer, pH 7.4). The rate of NADH oxidation was determined by following the decrease in absorbance at 340 nm for 3 min at 30-s interval using a spectrophotometer (Thermo-Spectronic).

Cell Cycle Analysis

Cell cycle distribution was assayed by determining DNA content. Cells were treated with IN and CN particles for 48 h. After exposure, cells were fixed in 3% (w/v) paraformaldehyde for 10 min, permeabilized on ice in phosphate buffer saline-0.5% Triton X-100 for 15 min, washed and resuspended in 0.5 ml of phosphate buffer saline containing 1% FBS, 1 mg/ml RNaseA, and 50 µg/ml propidium iodide. The samples were incubated for 30 min at 37°C. The data were acquired and analyzed on FACS-Calibur flow cytometer (Becton-Dickinson LSR II, San Jose, CA) using Cell Quest 3.3 software.

Measurement of Reactive Oxygen Species

For the measurement of ROS generation, cells were cultured in 12-well plate. The production of intracellular ROS was measured using 2,7-dichlorofluorescein diacetate (DCFH-DA) (Wang and Joseph, 1999). The DCFH-DA passively enters the cell where it reacts with ROS to form the highly fluorescent compound dichlorofluorescein (DCF). Briefly, 10 mM DCFH-DA stock solution (in methanol) was diluted in culture medium without serum or other additive to yield a 100 µM working solution. After exposure to IN and CN particles, cells were washed twice with HBSS and then incubated in 1 mL working solution of DCFH-DA at 37°C for 30 min. Cells were lysed in alkaline solution and centrifuged at 3000 rpm for 10 min. Then, a 200 µL supernatant (from 12-well plate) was transferred to the fresh well of black 96-well plates and fluorescence was measured using at 485 nm excitation and 520 nm emission using a microplate reader (FLUOstar-Omega). The values of ROS were expressed as percent of fluorescence intensity relative to controls.

Membrane Lipid Peroxidation Assay

The extent of membrane lipid peroxidation (LPO) was estimated by measuring the formation of thiobarbituric acid reactive species (TBARS) using the method of Ohkawa et al. (1979). Briefly, cells were cultured in 75 cm² culture flask and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. After the treatment, a 200 µL of cell suspension was mixed with 800 µL of LPO assay cocktail containing TBA (0.4%, w/v), sodium dodecyl sulphate (0.5%, w/v), and acetic acid (5 %, v/v). Reaction mixture was then incubated at 95°C for 1 h. After cooling to room temperature the reaction mixture was centrifuged at 5000 rpm for 5 min. The absorbance of the supernatants was read at 532 nm against the standard. The amount of TBARS was expressed as nmol/mg protein.

Intracellular Glutathione Assay

Intracellular GSH was quantified using the method of Hissin and Hilf (1976). Briefly, cells were cultured in 75 cm² culture flask and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. After the exposure completed, cells were lysed in 20 mM Tris (pH 7.0) and the centrifuged at 10,000 rpm for 10 min at 4°C. Further, protein of the supernatant was precipitated using 1% perchloric acid and again centrifuged at 10,000 rpm for 5 min at 4°C to get supernatant. Then 20 µL of supernatant was mixed with 160 µL of 0.1M potassium phosphate-5 mM EDTA buffer (pH 8.3) and 20 µL *O*-phthalaldehyde (1 mg/mL in methanol) in a black 96-well plate. After 2 h of incubation at room temperature in the dark, fluorescence was measured at emission wavelength of 460 nm and excitation wavelength of 350 nm. The amount of GSH was expressed as nmol GSH/mg protein.

Antioxidant Enzymes Activity Assay

Cells were cultured in 75 cm² culture flask and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. After the exposure completed, cells were harvested in ice-cold phosphate buffer saline and washed twice with phosphate buffer saline at 4°C. The cell pellets were then lysed in cell lysis buffer. Following centrifugation (10,000 rpm for 10 min 4°C) the supernatant (i.e. cell lysate) was maintained on ice until assayed for activity of superoxide dismutase (SOD) and catalase (CAT) enzymes. The total SOD was determined using pyrogallol assay following the procedure described by Marklund and Marklund (1974), based on the competition between pyrogallol oxidation by superoxide radicals and superoxide dismutation by SOD, and spectrophotometrically read at 420 nm. The amount of SOD inhibiting the reaction rate by 50% in the given assay conditions was defined as one

unit of SOD. The results were expressed as units/min/mg protein.

CAT activities were assayed by the method described by Claiborne (1985). One unit of CAT activity is defined as the amount of enzyme that decomposes 1 µmol H₂O₂/min. CAT activities were given as µmol H₂O₂ decomposed/min/mg protein.

DNA Ladder Assay

Cells were cultured in 6-well plate and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. At the end of exposure DNA was extracted using an apoptotic DNA Ladder Kit (Roche, Cat# 11835246001). The extracted DNA was then evaluated on a 1% agarose gel using ethidium bromide. DNA fragmentation pattern was documented by a gel documentation system.

Assay of Caspase-3 Enzyme

Cells were cultured in 6-well plate and exposed to IN and CN particles at the concentration of 200 µg/mL for 48 h. Activity of caspase-3 enzyme was determined using a standard fluorometric microplate assay (Walsh et al., 2008) with some modifications. A reaction mixture containing 30 µL of cell lysate, 20 µL of Ac-DEVD-AFC (caspase-3 substrate), and 150 µL of protease reaction buffer (50 mM Hepes, 1 mM EDTA, and 1 mM DTT), pH 7.2, was incubated for 15 min. Fluorescence of reaction mixture was measured at 5 min interval for 15 min at excitation/emission wavelengths of 430/535 nm using a microplate reader (FLUOstar-Omega). A standard of 7-amido-4-trifluoromethylcoumarin (AFC) ranging from 5 to 15 µM was prepared and its fluorescence was recorded for calculation of caspase-3 activity in terms of pmol AFC released/min/mg protein.

Estimation of Protein

The protein content was measured by the method of Bradford (1976) using Bradford reagent (Sigma-Aldrich, St. Louis, MO), along with bovine serum albumin as standard.

Statistics

All the data represented in this study are means ± SD of three identical experiments made in three replicate. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Significance was ascribed at $p < 0.05$. All analyses were conducted using the Prism software package (GraphPad Software).

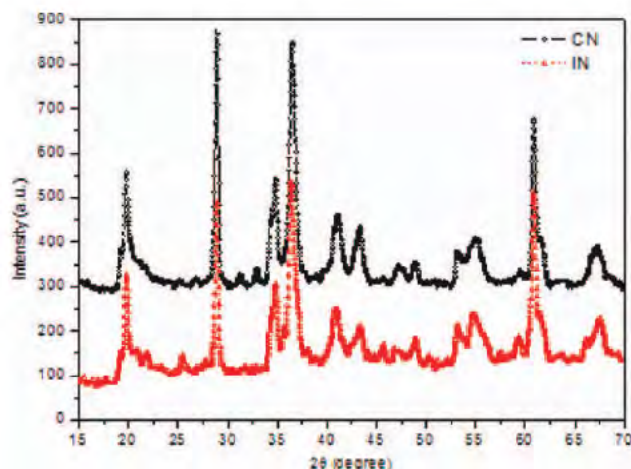


Fig. 1. XRD pattern of two types of nanotalc particles. IN; indigenous nanotalc particles, CN; commercial nanotalc particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS

Characterization of IN and CN Particles

Characterization of IN and CN particles was performed using a combination of XRD, TEM, DLS, zeta-potential, and BET in order to provide clear insight into crystalline nature, morphology, particle size, surface property, and chemical composition. These properties are necessary for a better understanding of nanotoxicology.

Figure 1 represents the XRD pattern of IN and CN particles. Image clearly exhibits that the crystalline nature of both IN and CN particles were same. The average size of nanocrystals calculated from the XRD results using Scherrer's equation (Patterson, 1939) was found to be 93 and 89 nm for IN and CN particles, respectively. Figure 2(A,B) show the typical TEM images of IN and CN particles, respectively. Images show that particles are aggregated. We never found small independent crystals in the TEM images.

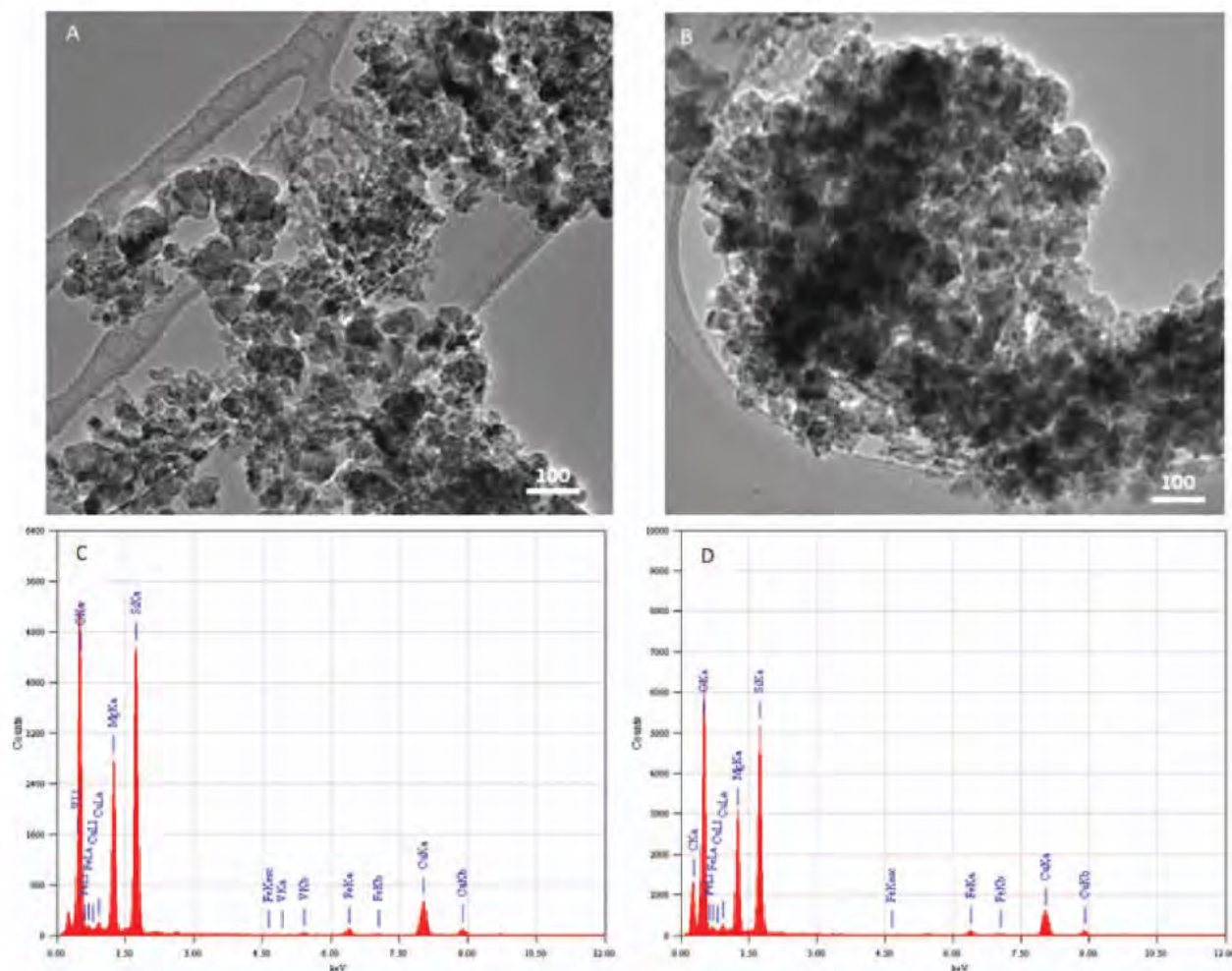


Fig. 2. TEM characterization of nanotalc particles. (A) FETEM of indigenous nanotalc particles, (B) FETEM of commercial nanotalc particles, (C) EDS spectrum of indigenous nanotalc particles, and (D) EDS spectrum of commercial nanotalc particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 1. Physicochemical properties of two types of nanotalc particles

	Indigenous Nanotalc (IN)	Commercial Nanotalc (CN)
Average XRD size (nm)	93	89
Average TEM size (nm)	94	91
Surface area (m ² /g)	15.4	15.7
Hydrodynamic size (nm)		
Distilled water	782	735
Cell culture medium	671	643
Zeta potential (mV)	20.3	20.8
Iron content (%) ^a	0.19	0.08

^aThis information is obtained from our previous publication (Akhtar et al., 2010a).

The average TEM size of IN and CN particles were 94 and 91 nm, respectively, which were consistent with the value observed by XRD. The EDS spectra of IN and CN particles are given in Figure 2(C,D), respectively. The presence of Cu and C signals was from the carbon-coated-copper TEM-grid. Presence of iron peaks in both IN and CN particles are in agreement with our previous reports where atomic absorption spectroscopy data showed that 0.19% and 0.08% of iron present in IN and CN particles, respectively (Akhtar et al., 2010 a). The specific surface area of IN and CN particles determined by BET was 15.4 and 15.7 m²/g respectively.

The physicochemical properties of IN and CN particles are listed in Table 1. All the data from XRD, electron microscopy, and associated techniques was obtained under high vacuum and constitutes the size, morphology, and composition analysis characteristics of nanotalc particles. However, once the nanotalc particles were introduced aqueous media, the sizes changed to approximately 5 to 10 times of the primary size. The average hydrodynamic size of IN and CN particles in distilled water was 782 nm and 735 nm while in cell culture media was 671 and 643 nm, respectively. The higher size of IN and CN particles in aqueous state as compared to XRD and TEM results was due to the tendency of particles to aggregate in the aqueous state. This finding is supported by other investigators (Murdock et al., 2008) and has been briefly discussed in our previous publications (Ahamed et al., 2010a,b). The tendency of particles to form aggregates depends strongly on the surface charge. The particle charge, determined as zeta-potential by laser doppler velocimetry (LDV) was 20.3mV and 20.8 for IN and CN, respectively.

IN and CN Particles Induced Cytotoxicity

We examined the cell viability (MTT assay) and membrane damage (LDH leakage) as cytotoxicity end points. MTT results demonstrated that both IN and CN particles induced significant reduction in cell viability. The MTT reduction

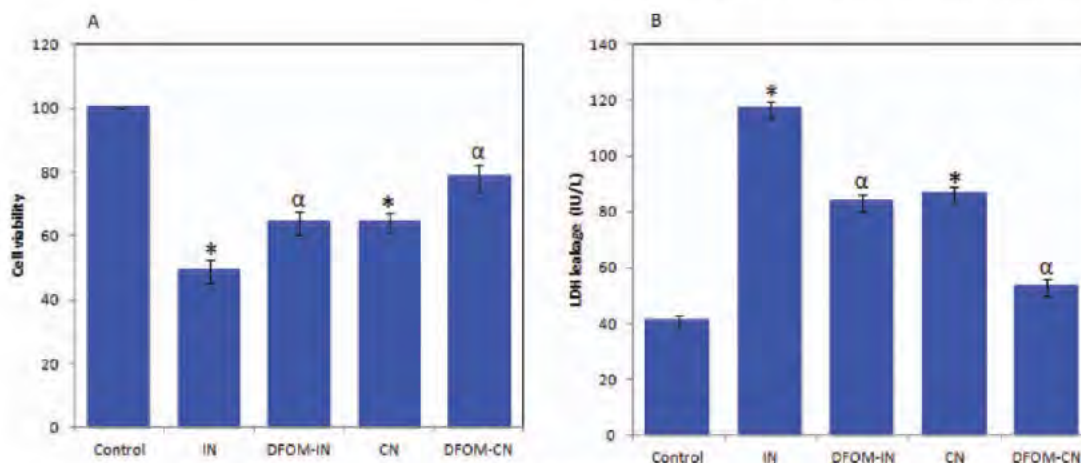


Fig. 3. Comparative effects of nanotalc particles and iron-chelated nanotalc particles on cell viability and LDH release in human lung epithelial A549 cells. Cells were treated with two types of nanotalc particles at the concentration of 200 μ g/mL for 48 h. Iron chelator deferoxamine mesylate (DFOM) was co-exposed with nanotalc particles. At the end of treatment MTT and LDH assays were determined as described in materials and methods. (A) MTT assay and (B) LDH assay. Data represented are mean \pm SD of three identical experiments made in three replicates. *Statistically significant difference in cell viability reduction and LDH release as compared with the controls ($p < 0.05$ for each). ^aIron chelation by DFOM significantly reduces the cytotoxicity caused by nanotalc particles ($p < 0.05$ for each). IN; indigenous nanotalc particles, CN; commercial nanotalc particles, DFOM-IN; indigenous nanotalc particles treated with DFOM, DFOM-CN; commercial nanotalc particles treated with DFOM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

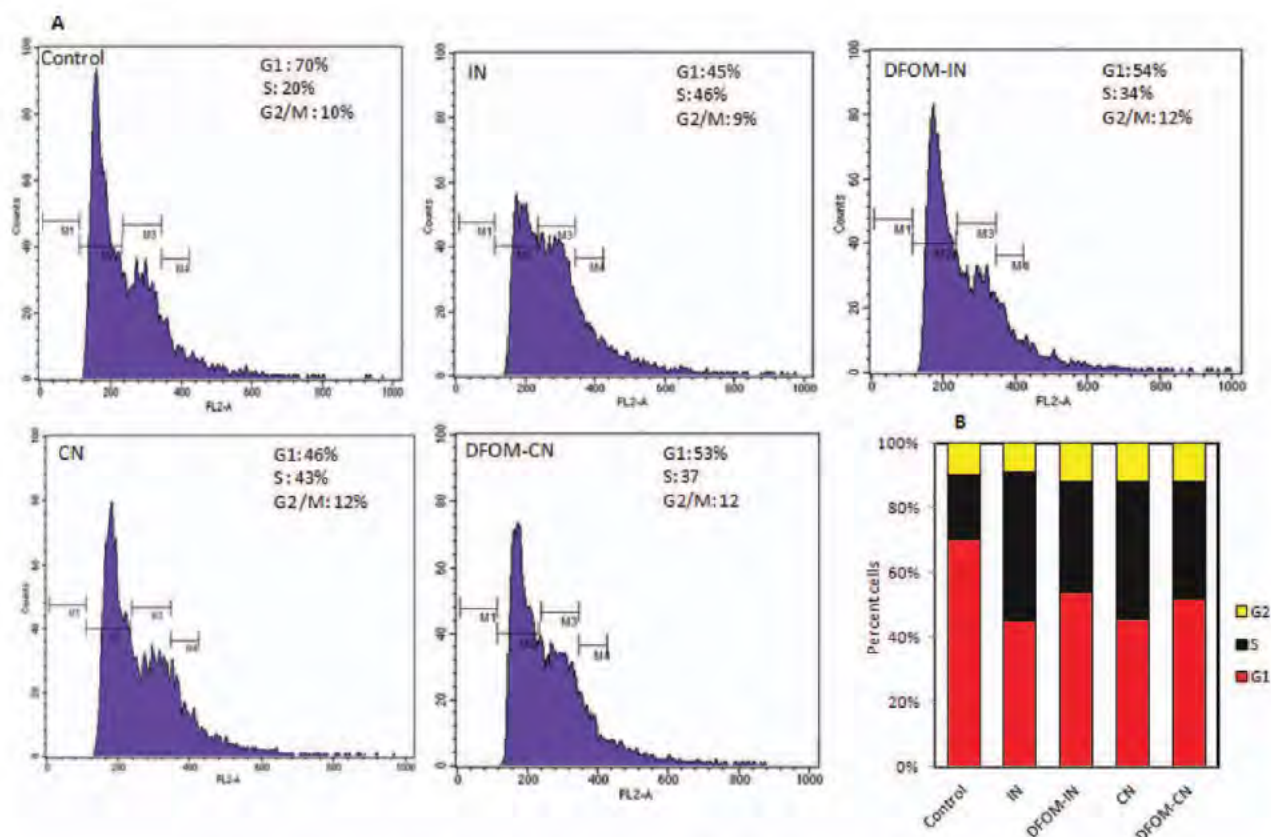


Fig. 4. Comparative effects of nanotalc particles and iron-chelated nanotalc particles on cell cycle in human lung epithelial A549 cells. Cells were treated with two types of nanotalc particles at the concentration of 200 $\mu\text{g/mL}$ for 48 h. Iron chelator deferoxamine mesylate (DFOM) was coexposed with nanotalc particles. At the end of treatment cell cycle was analyzed as described in materials and methods. (A) Raw data generated by flow cytometric analysis of selected representative samples. The y-axis denotes cell count and the x-axis represents DNA content. M1, M2, M3, and M4 represent the SubG1, G1, S, and G2/M phase, respectively. (B) Percent of the distribution of cells in the G1, S, and G2/M phase of cell cycle. IN; indigenous nanotalc particles, CN; commercial nanotalc particles, DFOM-IN; indigenous nanotalc particles treated with DFOM, DFOM-CN; commercial nanotalc particles treated with DFOM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed after 48 h at the concentration of 200 $\mu\text{g/mL}$ was 49% and 64% for IN and CN particles, respectively [Fig. 3(A)]. Both IN and CN particles were also found to induce LDH leakage in A549 cells [Fig. 3(B)]. To determine whether our observed cytotoxicity was due iron content, we treated both IN and CN particles with an iron chelator DFOM and tested the cytotoxic effect of chelated nanotalc particles in A549 cells. Results showed that iron chelated IN and CN particles induce less cytotoxicity than those of non-chelated one (Fig. 3).

IN and CN Particles Induced Cell Cycle Changes

Alteration in the cell cycle phases by IN and CN particles in A549 cells are shown in Figure 4. Both IN and CN par-

ticles induced significant S phase arrest. The S phase was 20% in the control. It was changed to 46% and 43% in the cells treated with IN and CN particles respectively. However, iron chelated IN and CN particles exert less effect on cell cycle arrest than those of nonchelated IN and CN particles.

IN and CN Particles Induced Oxidative Stress

ROS generation leads to oxidative damage, which has been reported to be one of the important mechanisms of nanoparticles toxicity (Ahamed et al., 2010c; Ahamed et al., 2011a,b). The potential of IN and CN particles to induce oxidative stress was examined by measuring the ROS, LPO, GSH, SOD, and CAT in A549 cells. Results showed that both IN and CN particles induced the

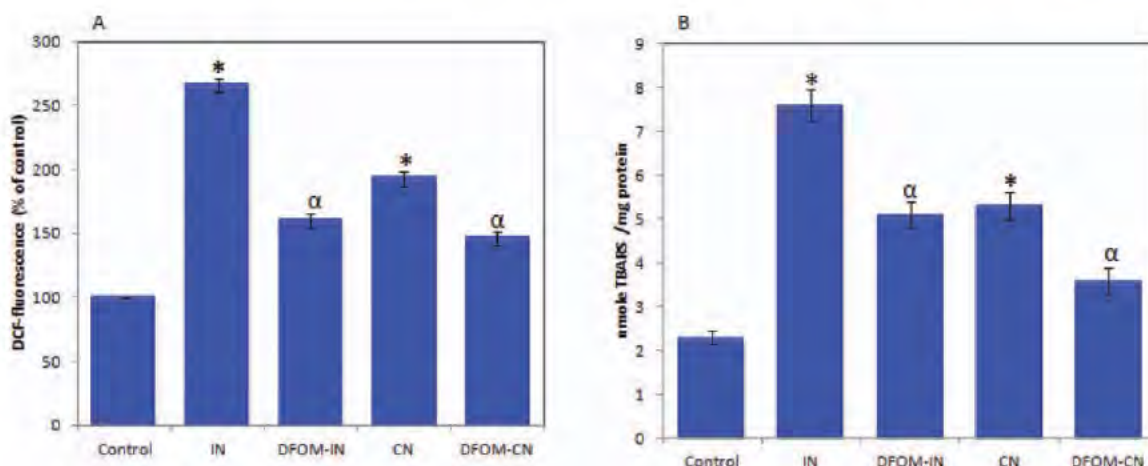


Fig. 5. Comparative effects of nanotalc particles and iron-chelated nanotalc particles on oxidant generations in human lung epithelial A549 cells. Cells were treated with two types of nanotalc particles at the concentration of 200 $\mu\text{g/mL}$ for 48 h. Iron chelator deferoxamine mesylate (DFOM) was coexposed with nanotalc particles. At the end of treatment ROS and LPO levels were determined as described in materials and methods. (A) ROS and (B) LPO. Data represented are mean \pm SD of three identical experiments made in three replicates. *Statistically significant difference in ROS and LPO induction as compared with the controls ($p < 0.05$ for each). ^αIron chelation by DFOM significantly reduces the ROS and LPO induction caused by nanotalc particles ($p < 0.05$ for each). IN; indigenous nanotalc particles, CN; commercial nanotalc particles, DFOM-IN; indigenous nanotalc particles treated with DFOM, DFOM-CN; commercial nanotalc particles treated with DFOM. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://www.wileyonlinelibrary.com).]

intracellular ROS and LPO levels [Fig. 5(A,B)]. Nanotalc particles induced oxidative stress was further evidenced by depletion of GSH, SOD, and CAT [Fig. 6(A,B,C)]. Moreover, chelation of iron from IN and CN particles significantly reduced the oxidative stress due to these particles.

IN and CN Particles Induced Apoptosis

Apoptosis is executed by series of cysteine proteases known as caspases (Takadera and Ohyashiki, 2007; Tang et al., 2010). Caspase-9 activation is dependent on the release of cytochrome c from mitochondria to form the apoptosome which in turn activates caspase-3. In the present study, significant higher activity of caspase-3 enzyme was observed suggesting the involvement of caspase cascade in IN and CN particles induced apoptosis in A549 cells [Fig. 7(B)]. Figure 7(B) shows that in untreated cells, the DNA was intact whereas the cells treated with IN and CN particles had started apoptotic DNA fragmentation. Besides, iron chelation from IN and CN particles induced less DNA fragmentation as compared with the nonchelated particles.

Taken together, our data highlight the role of iron contaminant present in IN and CN particles in causing the cytotoxicity, oxidative stress, and apoptosis in human lung epithelial cells.

DISCUSSION

Characterization of physicochemical properties of nanoparticles has been suggested in the nanotoxicology research (Murdock et al., 2008; Li et al., 2011). Several parameters including shape, size, crystal structure, purity, hydrodynamic size, aggregation of particles, and aqueous stability have already been suggested (Nel et al., 2006; Yu et al., 2009). In this study, we employed XRD, TEM, EDS, BET, and DLS techniques to characterize the physicochemical properties of IN and CN particles. XRD and TEM results indicated that both IN and CN particles were crystalline, highly aggregated, and having the iron content as a contaminant. Aggregation and stability of nanoparticles in aqueous state are major concerns in nanotoxicity research. Both IN and CN particles were also aggregated in water and cell culture media as well. Zeta potential data also showed that the aqueous suspension of both IN and CN particles were not much stable in aqueous state. The hydrodynamic size of nanotalc particles was found to be approximately seven to eight times higher than those calculated from TEM and XRD. The higher size of nanoparticles in aqueous suspension as compared with XRD and TEM sizes might be due to the tendency of particles to aggregate in aqueous state. This finding is supported by other investigators (Bai et al., 2009) and has been briefly discussed in our previous publication (Ahamed et al., 2010b).

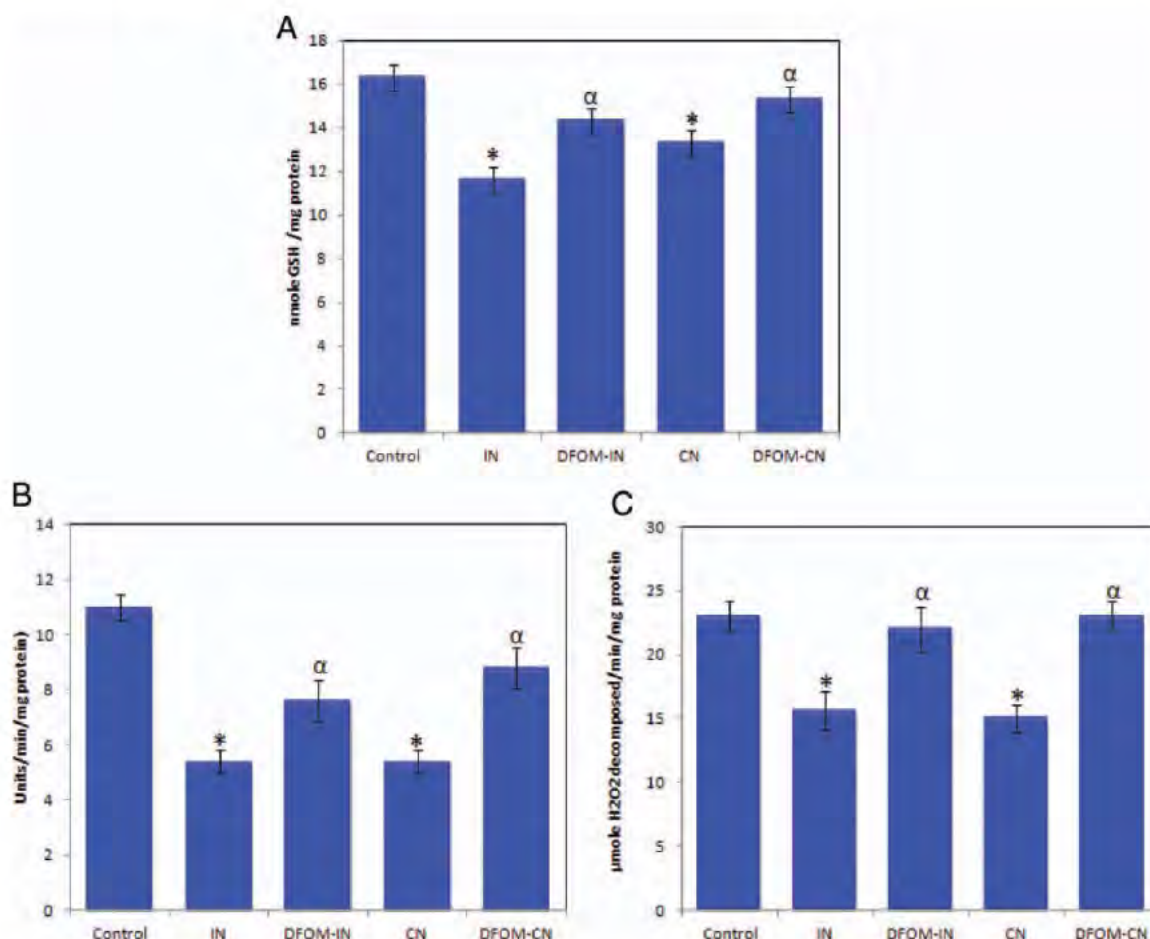


Fig. 6. Comparative effects of nanotalc particles and iron-chelated nanotalc particles on antioxidants reduction in human lung epithelial A549 cells. Cells were treated with two types of nanotalc particles at the concentration of 200 $\mu\text{g/mL}$ for 48 h. Iron chelator deferioxamine mesylate (DFOM) was coexposed with nanotalc particles. At the end of treatment GSH, SOD, and CAT levels were determined as described in materials and methods. (A) GSH, (B) SOD, and (C) CAT. Data represented are mean \pm SD of three identical experiments made in three replicates. *Statistically significant difference in GSH, SOD, and CAT reduction as compared to the controls ($p < 0.05$ for each). ^αIron chelation by DFOM significantly induces the GSH, SOD, and CAT depletion caused by nanotalc particles ($p < 0.05$ for each). IN; indigenous nanotalc particles, CN; commercial nanotalc particles, DFOM-IN; indigenous nanotalc particles treated with DFOM, DFOM-CN; commercial nanotalc particles treated with DFOM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In this study, we observed that IN and CN particles induced cell viability reduction and membrane damage in A549 cells. Both IN and CN particles also induced the cell cycle arrest in the S phase leading to apoptosis. In a previous study, S phase arrest was observed in mouse peritoneal macrophages (RAW264.7) exposed to silver nanoparticles (Park et al., 2010), and S phase arrest was also observed in human lung epithelial cells exposed to carbon black particles coated with benzo(a)pyrene (Mroz et al., 2007). Asharani et al. (2009) reported that starch-coated silver NPs induced G2/M phase arrest and DNA damage in human glioblastoma cells and fibroblasts. A perturbation of

the cell cycle preceded by a reduction in cell viability associated with accumulation of cells in S phase leading to cell death is typical of compounds inhibiting DNA synthesis (Binkova et al., 2000; Park et al., 2010).

Cellular integrity is affected by oxidative stress when the production of ROS overwhelms antioxidant defense mechanism (Halliwell and Gutteridge, 1990). Our results showed that both IN and CN particles induce oxidant levels and deplete the antioxidant levels in human lung epithelial (A549) cells. LPO and ROS were significantly higher while the antioxidant GSH was significantly lower in cells treated with IN and CN particles. Antioxidant enzymes SOD and

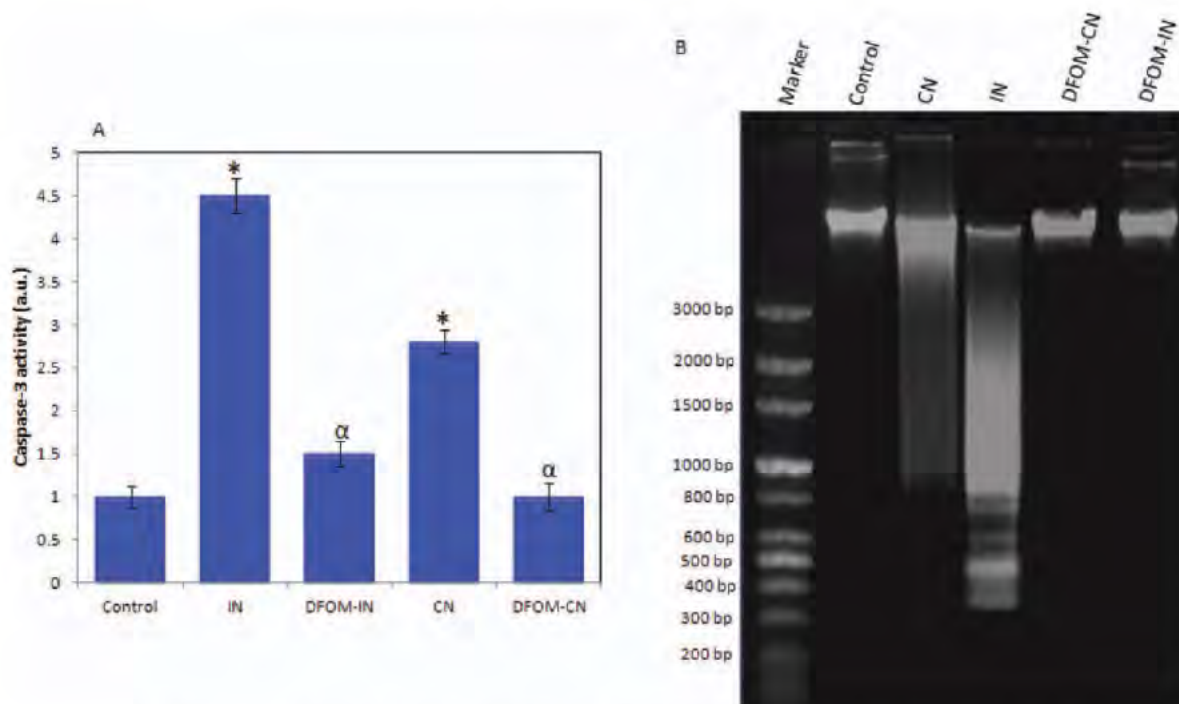


Fig. 7. Comparative effects of nanotalc particles and iron-chelated nanotalc particles on apoptotic markers in human lung epithelial A549 cells. Cells were treated with two types of nanotalc particles at the concentration of 200 $\mu\text{g/mL}$ for 48 h. Iron chelator deferoxamine mesylate (DFOM) was coexposed with nanotalc particles. At the end of treatment DNA ladder and caspase-3 activity were determined as described in materials and methods. (A) Caspase-3 activity. Data represented are mean \pm SD of three identical experiments made in three replicates. *Statistically significant difference in caspase-3 activation as compared with the controls ($p < 0.05$ for each). ^aIron chelation by DFOM significantly reduces the activity of caspase-3 by nanotalc particles ($p < 0.05$ for each). (B) Representative image of DNA fragmentation. IN; indigenous nanotalc particles, CN; commercial nanotalc particles, DFOM-IN; indigenous nanotalc particles treated with DFOM, DFOM-CN; commercial nanotalc particles treated with DFOM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CAT levels were also significantly lower in exposed cells. GSH constitutes the first line of the cellular defense mechanism against oxidative injury and is the major intracellular redox buffer in ubiquitous cell types (Meister, 1989). GSH acts as a cosubstrate in the GSH peroxidase-catalyzed reduction of hydrogen peroxide or lipid peroxides (Forman et al., 1997) leading to its depletion. Previous studies demonstrated that ROS generation following GSH depletion caused mitochondrial damage (Martensson et al., 1989), which has been implicated in apoptosis (Green and Reed, 1998). Enzymes such as SOD and CAT are meant for nullifying cellular oxidative stress. SOD catalyzes the dismutation of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2). CAT reduces hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2) (Claiborne, 1985).

The activity of caspase-3 enzyme was significantly higher in cells treated with IN and CN particles. Apoptotic DNA fragmentation was observed in cells exposed to IN

and CN particles. Caspases are activated in response to diverse cell death stimuli and ultimately dismantle the cell through restricted proteolysis of numerous cellular proteins that (Timmer and Salvesen, 2007). The activated caspase-3 is capable of autocatalysis as well as cleaving and activating other members of the caspase family, leading to rapid and irreversible apoptosis (Wang et al., 1996). Our previous studies also reported that different types of nanoparticles have potential to induce apoptosis in different kind of cells (Ahamed et al., 2010a; 2010b; 2010c; Ahamed et al., 2010b,c; 2011a).

In the toxicity mechanism of minerals, the iron content has been a key factor. In the present study, EDS analysis showed the presence of iron contamination in both IN and CN particles. These results are in agreement with our previous report where atomic absorption spectroscopy showed the presence of 0.19% and 0.08% of iron in IN and CN particles respectively (Akhtar et al., 2010a). Iron-dependent

ROS generation from fibers results in the generation of hydroxyl radicals through the Fenton reaction and the Haber-Weiss cycle. Iron-dependent ROS generation requires redox cycling of iron and does not necessarily require H_2O_2 or ROS (Halliwell and Gutteridge, 1990). The differential amount of iron present in the two types of nanotalc particles prompted us to investigate the role of iron by sequestering them with an iron chelator, deferoxamine mesylate (DFOM). Sequestering of redox active iron from IN and CN particles by DFOM caused significantly less cytotoxicity, oxidative stress, and genotoxicity than those of the nonchelated IN and CN particles. Similarly, incubation of crocidolite or chrysotile fibers overnight with deferoxamine (5 mM) to inactivate iron catalyzed oxygen radical production also significantly decreased asbestos-induced apoptosis (Broaddus et al., 1996). The role of iron in minerals such as asbestos or silica has been well reported in inflammation and carcinogenesis (Ghio et al., 1992; Hardy and Aust, 1995). Zastawny et al. (1995) have reported on DNA base modifications and membrane damage in cultured mammalian cells treated with iron itself. Similarly, intracellular iron was found to play a critical role in hydrogen peroxide-induced DNA damage (Barboudi et al., 2001). It is also worth to mention that IN particles caused higher toxicity to A549 cells than those of CN particles. This might be due to higher amount of iron present in IN particles (0.19%) as compared with the CN particles (0.08%).

In conclusion, both IN and CN particles significantly induced cytotoxicity, oxidative stress, and apoptosis in human lung epithelial cells. Further, chelation of iron from IN and CN particles by deferoxamine mesylate treatment caused significantly less toxicity as compared to non-chelated IN and CN particles. Therefore, iron content plays a significant role in the toxicity of IN and CN particles, which may be mediated through ROS generation and oxidative stress. This study suggests that one must be very careful regarding the metal impurities like iron present in nanotalc particles before commercial and industrial applications.

REFERENCES

- Ahamed M, AlSalhi MS, Siddiqui MKJ. 2010a. Silver nanoparticle applications and human health. *Clin Chim Acta* 411:1841–1848.
- Ahamed M, Posgai R, Gorey TJ, Nielsen M, Hussain S, Rowe J. 2010b. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in *Drosophila melanogaster*. *Toxicol Appl Pharmacol* 242:263–269.
- Ahamed M, Siddiqui MA, Akhtar MJ, Ahmad I, Pant AB, Alhaddad HA. 2010c. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. *Biochem Biophys Res Commun* 396:578–583.
- Ahamed M. 2011a. Toxic response of nickel nanoparticles in human lung epithelial A549 cells. *Toxicol In Vitro* 25:930–936.
- Ahamed M, Akhtar MJ, Raja M, Ahmad I, Siddiqui MKJ, AlSalhi MS, Alrokayan SA. 2011b. ZnO nanorod induced apoptosis via p53, survivin and bax/bcl 2 pathways mediated by oxidative stress in human alveolar adenocarcinoma cells. *Nano medicine:NBM* 7:904–913.
- Ahamed M, Akhtar MJ, Siddiqui MA, Ahmad J, Musarrat J, AlKhedhairi AA, AlSalhi MS, Alrokayan SA. 2011c. Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. *Toxicology* 283:101–108.
- Akhtar S, Shukla D, Kumar V. 2008. Studies on effect of nanotalc filler on nucleation, crystal morphology and crystallization behaviour of semi crystalline plastics. *Solid State Phenomena* 136:161–174.
- Akhtar MJ, Kumar S, Murthy RC, Ashquin M, Khan MI, Patil G, Ahmad I. 2010a. The primary role of iron mediated lipid peroxidation in the differential cytotoxicity caused by two varieties of talc nanoparticles on A549 cells and lipid peroxidation inhibitory effect exerted by ascorbic acid. *Toxicol In Vitro* 24:1139–1147.
- Akhtar MJ, Ahamed M, Kumar S, Siddiqui H, Patil G, Ashquin M, Ahmad I. 2010b. Nanotoxicity of pure silica mediated through oxidant generation rather than glutathione depletion in human lung epithelial cells. *Toxicology* 276:95–102.
- AshaRani PV, Mun G, Hande MP, Valiyaveetil S. 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3:279–290.
- Aung W, Hasegawa S, Furukawa T, Saga T. 2007. Potential role of ferritin heavy chain in oxidative stress and apoptosis in human mesothelial and mesothelioma cells: Implications for asbestos induced oncogenesis. *Carcinogenesis* 28:2047–2052.
- Balamurugan GP, Maiti SN. 2010. Effects of nanotalc inclusion on mechanical, microstructural, melt shear rheological, and crystallization behavior of polyamide 6 based binary and ternary nanocomposites. *Polym Eng Sci* 50:1978–1993.
- Bai W, Zhang Z, Tian W, He X, Ma Y, Zhao Y, Chai Z. 2009. Toxicity of zinc oxide nanoparticles to zebrafish embryo: A physicochemical study of toxicity mechanism. *J Nanopart Res* 12:1645–1654.
- Barboudi A, Doulias PT, Zhu BZ, Frei B, Galaris D. 2001. Intracellular iron, but not copper, plays a critical role in hydrogen peroxide induced DNA damage. *Free Radical Biol Med* 31:490–498.
- Binkova B, Giguère Y, Rossner Jr P, Dost M, Srm RJ. 2000. The effect of dibenzo[a, l]pyrene and benzo[a]pyrene on human diploid lung fibroblasts: The induction of DNA adducts, expression of p53 and p21(WAF1) proteins and cell cycle distribution. *Mutat Res* 471:57–70.
- Bizi M, Flament MP, Leterne P, Baudet G, Gayot A. 2003. Relation between structural characteristics of talc and its properties as an antisticking agent in the production of tablets. *Eur J Pharm Sci* 19:373–379.
- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 72:248–254.
- Broaddus VC, Yang L, Scavo LM, Ernst JD, Boylan AM. 1996. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. *J Clin Invest* 98:2050–2059.

- Buz'Zard AR, Lau BH. 2007. Pycnogenol reduces talc induced neoplastic transformation in human ovarian cell cultures. *Phyt other Res* 21:579 286.
- Carretero MI. 2002. Clay minerals and their beneficial effects upon human health. *Appl Clay Sci* 21:155 163.
- Claiborne A. 1985. Catalase activity. In: Greenwald RA, editor. *Handbook of Methods for Oxygen Radical Research*. CRC Press Inc. pp283 284.
- Forman HJ, Liu R, Tian L. 1997. Glutathione cycling in oxidative stress. In: Clerch LB, Massaro DJ, editors. *Oxygen, Gene Expression, and Cellular Function: Lung Biology in Health and Disease*, Vol. 105. New York: Marcel Dekker. pp99 112.
- Gates MA, Tworoger SS, Terry KL, Titus Ernstoff L, Rosner B, De Vivo I, Cramer DW, Hankinson SE. 2008. Talc use, variants of the GSTM1, GSTT1, and NAT2 genes, and risk of epithelial ovarian cancer. *Cancer Epidemiol Biomark Prev* 17: 2436 2444.
- Green DG, Reed JC. 1998. Mitochondria and apoptosis. *Science* 281:1309 1312.
- Halliwell B, Gutteridge JM. 1990. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol* 186:1 85.
- Hardy JA, Aust AE. 1995. Iron in asbestos chemistry and carcinogenicity. *Chem Rev* 95:97 118.
- Harlow BL, Hartge PA. 1995. A review of perineal talc exposure and risk of ovarian cancer. *Regul Toxicol Pharmacol* 21: 254 260.
- Hissin PJ, Hilf R. 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal Biochem* 74:214 226.
- Jaynes WF, Zartman RE. 2005. Origin of talc, iron phosphates, and other minerals in biosolids. *Soil Sci Soc Am J* 69: 1047 1056.
- Langseth H, Hankinson SE, Siemiatycki J, Weiderpass E. 2008. Perineal use of talc and risk of ovarian cancer. *J Epidemiol Community Health* 62:358 360.
- Li Y, Sun L, Jin M, Du Z, Liu X, Guo C, Li Y, Huang P, Sun Z. 2011. Size dependent cytotoxicity of amorphous silica nanoparticles in human hepatoma HepG2 cells. *Toxicol in Vitro* 25:1343 1352.
- Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Euro J Biochem* 47:469 474.
- Martensson J, Jain A, Frayer W, Meister A. 1989. Glutathione metabolism in the lung: inhibition of its synthesis leads to lamellar body and mitochondrial defects. *Proc Natl Acad Sci USA* 86:5296 5300.
- Meister A. 1989. Taniguchi N, Higashi T, Sakamoto Y, Meister A, eds. In: *Glutathione Centennial: Molecular Properties and Clinical Applications*. New York, NY: Academic Press.
- Mroz RM, Schins RP, Li H, Drost EM, Macnee W, Donaldson K. 2007. Nanoparticle carbon black driven DNA damage induces growth arrest and AP 1 and NFkappaB DNA binding in lung epithelial A549 cell line. *J Physiol Pharmacol* 58(Suppl 5):461 470.
- Mossman T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 65:55 63.
- Murdock RC, Braydich Stolle L, Schrand AM, Schlager JJ, Husain SM. 2008. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. *Toxicol Sci* 101:239 253.
- National Toxicology Program. 1993. *NTP Toxicology and Carcinogenesis Studies of Talc (Non Asbestiform) in Rats and Mice (Inhalation Studies)*, Vol. 421. pp1 287.
- Nel A, Xia T, Madler L, Li N. 2006. Toxic potential of materials at the nanolevel. *Science* 311:622 627.
- Nkoumbou C, Villieras F, Njopwouo D, Ngoune CY, Barres O, Pelletier M, Razafitianamaharavo A, Yvon J. 2008. Physicochemical properties of talc ore from three deposits of Lamal Pougue area (Yaounde Pan African Belt, Cameroon), in relation to industrial uses. *Appl Clay Sci* 41:113 132.
- Ohkawa H, Ohisi N, Yagi Y. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351 358.
- Park EJ, Yi J, Kim Y, Choi K, Park K. 2010. Silver nanoparticles induce cytotoxicity by a Trojan horse type mechanism. *Toxicol In Vitro* 24:872 878.
- Patterson AL. 1939. The Scherrer formula for x ray particle size determination. *Phys Rev* 56:978 782.
- Pérez Maqueda LA, Duran A, Pérez Rodriguez JL. 2005. Preparation of submicron talc particles by sonication. *Appl Clay Sci* 28:245 255.
- Persson HL, Yu Z, Tirosh O, Eaton JW, Brunk UT. 2003. Prevention of oxidant induced cell death by lysosomotropic iron chelators. *Free Radic Biol Med* 34:1295 1305.
- Petit S, Martin F, Wiewora A, De Parseval P, Decarreau A. 2004. Crystal chemistry of talc: A near infrared (NIR) spectroscopy study. *Am Mineral* 89:319 326.
- Sakthivel S, Pitchumani B. 2011. Production of nano talc material and its applicability as filler in polymeric nanocomposites. *Particle Sci Technol* 29:441 449.
- Takadera T, Ohyashiki T. 2007. Caspase dependent apoptosis induced by calcineurin inhibitors was prevented by glycogen synthase kinase 3 inhibitors in cultured rat cortical cells. *Brain Res* 1133:20 26.
- Tang X, Guo Y, Nakamura K, Huang H, Hamblin M, Chang L, Villacorta L, Yin K, Ouyang JH, Zhang J. 2010. Nitroalkenes induce rat aortic smooth muscle cell apoptosis via activation of caspase dependent pathways. *Biochem Biophys Res Commun* 397:239 244.
- Timmer JC, Salvesen GS. 2007. Caspase substrates. *Cell Death Differ* 14:66 72.
- Wang H, Joseph JA. 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic Biol Med* 27:612 616.
- Wang X, Zelenski NG, Yang J, Sakai J, Brown MS, Goldstein JL. 1996. Cleavage of sterol regulatory element binding proteins (SREBPs) by cyp32 during apoptosis. *EMBO J* 15:1012 1020.
- Welder AA, Grant R, Bradlaw J, Acosta D. 1991. A primary culture system of adult rat heart cells for the study of toxicologic agent. *In Vitro Cell Dev Biol* 27:921 926.
- Wild P. 2006. Lung cancer risk and talc not containing asbestos form fibres: A review of the epidemiological evidence. *Occup Environ Med* 63:4 9.

406 AKHTAR ET AL.

- Wroblewski F, LaDue JS. 1955. Lactate dehydrogenase activity in blood. *Proc Soc Exp Biol Med* 90:210–213.
- Yu KO, Grabinski CM, Schrand AM, Murdock RC, Wang W, Gu B, Schlager JJ, Hussain SM. 2009. Toxicity of amorphous silica nanoparticles in mouse keratinocytes. *J Nanopart Res* 11:15–24.
- Zastawny TH, Altman SA, Randers Eichhorn L, Madurawe R, Lumpkin JA, Dizdaroglu M, Rao G. 1995. DNA base modifications and membrane damage in cultured mammalian cells treated with iron ions. *Free Radic Biol Med* 18:1013–1022.
- Zhang J, Zhang T, Ti X, Shi J, Wu C, Ren X, Yin X. 2010. Curcumin promotes apoptosis in A549/DDP multidrug resistant human lung adenocarcinoma cells through an miRNA signaling pathway. *Biochem Biophys Res Commun* 6:1–6.

Exhibit 93

Alterations in Gene Expression in Human Mesothelial Cells Correlate with Mineral Pathogenicity

Arti Shukla^{1*}, Maximilian B. MacPherson^{1*}, Jedd Hillegass¹, Maria E. Ramos-Nino¹, Vlada Alexeeva¹, Pamela M. Vacek², Jeffrey P. Bond³, Harvey I. Pass⁴, Chad Steele⁵, and Brooke T. Mossman¹

Departments of ¹Pathology, ²Medical Biostatistics, and ³Microbiology and Molecular Genetics, University of Vermont College of Medicine, Burlington, Vermont; ⁴Department of Cardiothoracic Surgery, NYU Langone Medical Center, New York, New York; and ⁵Department of Medicine, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama

Human mesothelial cells (LP9/TERT 1) were exposed to low and high (15 and 75 $\mu\text{m}^2/\text{cm}^2$ dish) equal surface area concentrations of crocidolite asbestos, nonfibrous talc, fine titanium dioxide (TiO_2), or glass beads for 8 or 24 hours. RNA was then isolated for Affymetrix microarrays, GeneSifter analysis and QRT PCR. Gene changes by asbestos were concentration and time dependent. At low nontoxic concentrations, asbestos caused significant changes in mRNA expression of 29 genes at 8 hours and of 205 genes at 24 hours, whereas changes in mRNA levels of 236 genes occurred in cells exposed to high concentrations of asbestos for 8 hours. Human primary pleural mesothelial cells also showed the same patterns of increased gene expression by asbestos. Nonfibrous talc at low concentrations in LP9/TERT 1 mesothelial cells caused increased expression of 1 gene Activating Transcription Factor 3 (ATF3) at 8 hours and no changes at 24 hours, whereas expression levels of 30 genes were elevated at 8 hours at high talc concentrations. Fine TiO_2 or glass beads caused no changes in gene expression. In human ovarian epithelial (IOSE) cells, asbestos at high concentrations elevated expression of two genes (NR4A2, MIP2) at 8 hours and 16 genes at 24 hours that were distinct from those elevated in mesothelial cells. Since ATF3 was the most highly expressed gene by asbestos, its functional importance in cytokine production by LP9/TERT 1 cells was assessed using siRNA approaches. Results reveal that ATF3 modulates production of inflammatory cytokines (IL 1 β , IL 13, G CSF) and growth factors (VEGF and PDGF BB) in human mesothelial cells.

Keywords: mesothelioma; crocidolite asbestos; talc; titanium dioxide; gene profiling

A myriad of natural and synthetic fibers and particles, including nanomaterials, are being introduced into the workplace and environment, and *in vitro* screening tests on human cell types are needed to predict their toxicity and mechanisms of action, especially in target cells of disease. Asbestos is a group of well characterized fibrous minerals that are associated with the development of nonmalignant (asbestosis) and malignant (lung cancers, pleural, and peritoneal mesotheliomas) diseases in occupational cohorts (1–3), yet the molecular mechanisms of asbestos related diseases are poorly understood. Although it is widely acknowledged that fibrous geometry, surface and chemical composition, and durability are important features in the development

CLINICAL RELEVANCE

Results of work here suggest that transcriptional profiling can be used to reveal molecular events by mineral dusts that are predictive of their pathogenicity in mesothelioma.

of asbestos associated diseases, how these contribute to cell toxicity and transformation are unclear. Moreover, the early molecular events leading to injury by asbestos fibers and other pathogenic or innocuous particulates in human cells that may be targets for the development of disease remain enigmatic.

The objective of work here was to compare acute toxicity and gene expression profiles of crocidolite asbestos, the type of asbestos most pathogenic in the causation of human mesothelioma (3, 4), to nonfibrous talc, fine titanium dioxide (TiO_2), and glass beads in a contact inhibited, hTERT immortalized human mesothelial cell line (5). In comparative studies, we also evaluated toxicity of particulates and gene expression changes in a contact inhibited SV40 Tag immortalized human ovarian epithelial cell line (IOSE) (6). This cell type is not implicated in asbestos induced diseases, but is occasionally linked to inflammation and the development of ovarian cancer after use of talcum powder in the pelvic region, although such links are highly controversial (7).

Although most studies have evaluated the biological effects of particles and fibers on an equal mass or weight basis, the number, surface area, and reactivity of particulates at equal weight concentrations may be vastly different. Moreover, recent *in vitro* (8, 9) and *in vivo* (10–12), studies have confirmed that toxicity, oxidative stress, and inflammatory effects of ultrafine and other particles are related directly to surface area. For these reasons, and to avoid possible confounding alterations in gene expression or toxicity that might reflect or be masked in cells in different phases of the cell cycle, we introduced particulates at equal surface areas to confluent monolayers of human mesothelial (LP9/TERT 1) and human ovarian epithelial (IOSE) cells in a maintenance medium. Moreover, our studies included a nonfibrous talc sample and fine TiO_2 and glass particles, both traditionally used as nontoxic and nonpathogenic control particles in *in vitro* and animal experiments (reviewed in Refs. 13 and 14). Our studies provide novel insight into the early molecular events and responses occurring in human cells after exposure to asbestos and these materials.

MATERIALS AND METHODS

Human Mesothelial and Ovarian Epithelial Cell Cultures

Human mesothelial LP9/TERT 1 (LP9) cells, an hTERT immortalized cell line phenotypically and functionally resembling normal human mesothelial cells (5), were obtained from Dr. James Rheinwald (Dana Farber Cancer Research Institute, Boston, MA). Human pleural mesothelial cells (NYU474) were isolated surgically from

(Received in original form April 11, 2008 and in final form November 24, 2008)

* These authors contributed equally to this research.

This work was supported by NIEHS training grant T32ES007122 to B.T.M., a contract from EUROTALC and the Industrial Minerals Association of North America, and NCI P01 CA 114,047 (H.I.P. and B.T.M.).

Correspondence and requests for reprints should be addressed to Arti Shukla, Ph.D., Department of Pathology, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405. E-mail: Arti.Shukla@uvm.edu

This article contains microarray data which can be found as a repository using the accession number GSE14034.

Am J Respir Cell Mol Biol Vol 41, pp 114–123, 2009

Originally Published in Press as DOI: 10.1165/rcmb.2008-0146OC on December 18, 2008
Internet address: www.atsjournals.org

cancer free patients by Dr. Harvey Pass (New York University, New York, NY). Briefly, tissue sample $2 \times 2 \text{ cm}^2$ was harvested into saline solution and rinsed immediately with PBS (1 \times) and Dulbecco's modified Eagle's medium (DMEM) (1 \times). The tissue was then digested with 0.2% Collagenase type 1 (MP Biomedical Inc., Solon, OH) for 3 hours at 37°C. Finally, the digested tissue was scraped and cells collected were centrifuged for 5 minutes at $300 \times g$. The cell pellet thus obtained was resuspended in DMEM containing 10% fetal bovine serum (FBS) and 2% penicillin streptomycin, transferred into 6 well plate, and allowed to grow at 5% CO_2 and 37°C. Mesothelial cells were characterized by staining with calretinin antibody. An SV40 Tag immortalized, anchorage dependent human ovarian epithelial cell line (IOSE 398) (6) was a kind gift from Dr. Nelly Auersperg (Canadian Ovarian Tissue Bank, University of British Columbia, Vancouver, BC, Canada). LP9/TERT 1 cells were maintained in 50:50 DMEM/F 12 medium containing 10% FBS, and supplemented with penicillin (50 units/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), hydrocortisone (100 $\mu\text{g}/\text{ml}$), insulin (2.5 $\mu\text{g}/\text{ml}$), transferrin (2.5 $\mu\text{g}/\text{ml}$), and selenium (2.5 $\mu\text{g}/\text{ml}$). IOSE cells were maintained in 50:50 199/105 medium containing 10% FBS and 50 $\mu\text{g}/\text{ml}$ gentamicin. Cells at near confluence were switched to maintenance medium containing 0.5% FBS for 24 hours before particulate exposure. NYU474 cells were grown to near confluence in DMEM containing 10% FBS and supplemented with penicillin (50 units/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$).

Characterization of Mineral Preparations

The physical and chemical characterization of the NIEHS reference sample of crocidolite asbestos has been reported previously (15). The surface area of asbestos fibers and particles was measured using nitrogen gas sorption analysis to allow computation of identical amounts of surface areas of particulates to be added to cells. Fiber and particle size dimensions were determined by scanning electron microscopy (SEM) as described previously (16). In addition, talc was examined using field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The chemical composition, surface area, mean size, and source of each particulate preparation is presented in Table 1.

Introduction of Particulates to Cells

After sterilization under ultraviolet light overnight to avoid endotoxin and microbial contamination, particulates were suspended in HBSS at 1 mg/ml, sonicated for 15 minutes in a water bath sonicator, and triturated five times through a 22 gauge needle. This suspension was added to cells in medium.

SEM to Determine Particulate/Cell Interactions

Cells were grown on Thermoform plastic cover slips (Nalge Nunc International, Naperville, IL), exposed to particulates for 24 hours, and then processed for SEM as described previously (16). After samples were critical point dried, they were mounted on aluminum specimen stubs and dried before being sputter coated with gold and palladium in a Polaron sputter coater (Model 5100; Quorum Technologies, Guelph, ON, Canada) and examined on a JSM 6060 scanning electron microscope (JEOL USA, Inc., Peabody, MA).

Cell Viability Studies

After 24 hours, cells were collected with Accutase cell detachment reagent, and final cell suspensions in Accutase/complete medium/HBSS

were mixed with 0.4% trypan blue stain, which is retained by dead cells. After 5 minutes, unstained cells were counted using a hemocytometer to determine the total number of viable cells per dish.

Based on the results of cell viability studies, asbestos and nonfibrous talc were evaluated in LP9 mesothelial cells for changes in gene expression at both low and high concentrations (15 and 75 $\mu\text{m}^2/\text{cm}^2$ dish) at 8 hours, and at low concentrations of minerals (15 $\mu\text{m}^2/\text{cm}^2$ dish) at 24 hours. These concentrations did not cause morphologic or toxic cellular changes at these time points. Negative control groups included cells exposed to fine TiO_2 (15 $\mu\text{m}^2/\text{cm}^2$ dish) at 8 and 24 hours and glass beads (75 $\mu\text{m}^2/\text{cm}^2$) at 24 hours. In IOSE cells, gene expression of all particulates was evaluated at 75 $\mu\text{m}^2/\text{cm}^2$ at 8 and 24 hours, as preliminary experiments revealed that no significant changes in mRNA levels were observed at 15 $\mu\text{m}^2/\text{cm}^2$ dish of asbestos. In NYU474 human mesothelial cells, QRT PCR was used to validate a selected subset of gene expression changes identified by arrays in LP9/TERT 1 cells. Cells were exposed to 15 and 75 $\mu\text{m}^2/\text{cm}^2$ asbestos for 24 hours, and 8 genes highly expressed in LP9 cells were examined by QRT PCR (*see below*).

RNA Preparation

Total RNA was prepared using an RNeasy Plus Mini Kit according to the manufacturers' protocol (Qiagen, Valencia, CA), as previously described (17).

Affymetrix Gene Profiling

Microarrays were performed on samples from three independent experiments. All cell types, time points, and mineral types and concentrations were included in all three experiments. For each experiment, $n = 3$ dishes were pooled into one sample per treatment group. Each of the pooled samples was analyzed on a separate array (i.e., $n = 3$ arrays per condition [3 independent biological replicates]). All procedures were performed by the Vermont Cancer Center DNA facility using standard Affymetrix protocol as previously described (14, 17). Each probe array, Human U133A 2.0 (Affymetrix, Santa Clara, CA) was scanned twice (Hewlett Packard GeneArray Scanner, Palo Alto, CA), the images overlaid, and the average intensities of each probe cell compiled. Microarray data were analyzed using GeneSifter software (VizX Labs, Seattle, WA). This program used a "t test" for pairwise comparison and a Benjamini Hochberg test for false discovery rate (FDR 5%) to adjust for multiple comparisons. A 2 fold cutoff limit was used for analysis.

Quantitative Real Time PCR

Total RNA (1 μg) was reverse transcribed with random primers using the Promega AMV Reverse Transcriptase kit (Promega, Madison, WI) according to the recommendations of the manufacturer, as described previously (17). In NYU474 mesothelial cells, eight genes (*ATF3*, *SOD2*, *PTGS2*, *FOSB*, *TFPI2*, *PKD4*, *NR4A2*, and *IL 8*) most highly expressed in LP9 cells were evaluated using the $\Delta\Delta\text{Ct}$ method. Duplicate or triplicate assays were performed with RNA samples isolated from at least three independent experiments. The values obtained from cDNAs and hypoxanthine phosphoribosyl transferase (*hprt*) controls provided relative gene expression levels for the gene locus investigated. The primers and probes used to validate gene expression as observed in microarrays were purchased from Applied Biosystems (Foster City, CA).

TABLE 1. CHARACTERIZATION OF PARTICULATES

Name	Chemical Composition	Mean Surface Area \pm SE (m^2/g)	Mean Size (μm)*	Source
Crocidolite Asbestos	$\text{Na}_2\text{Fe}_3^{2+}\text{Fe}_2^{3+}\text{Si}_8\text{O}_{22}(\text{OH})_2$	14.97 ± 0.605	7.4×0.25	NIEHS Reference Sample
Talc (MP 10-52) [†]	$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$	16.03 ± 0.654	1.1	Barrett's Minerals, Inc.
Titanium Dioxide	TiO_2	9.02 ± 0.185	0.69	Fisher Scientific
Glass Beads	SiO_2	2.78 ± 0.215	2.06	Polysciences Inc.

* Length X width for crocidolite asbestos, and diameter for nonfibrous talc, TiO_2 , and glass beads.

[†] Although standard reference samples of asbestos and some particulates are available for use by the scientific community, reference samples of talc currently do not exist. For these reasons, the nonfibrous talc sample was also characterized for physical properties, particle size distribution (0.70 μm minimum to 1.20 μm maximum), and chemical/mineralogical (talc 95%, chlorite 4.5–5%, dolomite 0.3%) composition. For complete analysis or obtaining samples, please contact Brooke Mossman, Mark Ellis (markellis@ima-na.org), or Michelle Wyart at EUROTALC (mwyart@ima-europe.eu).

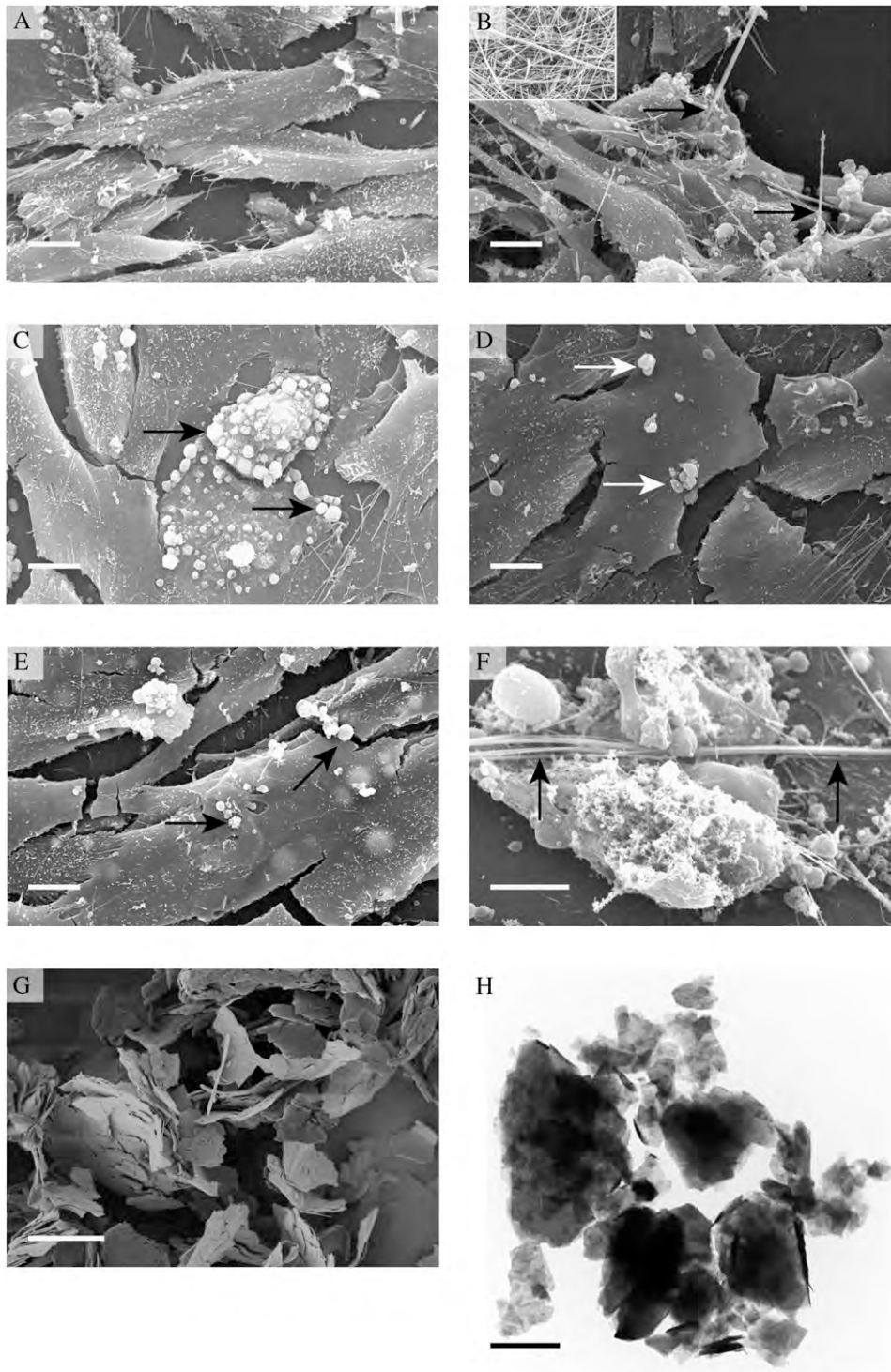


Figure 1. Interaction of fibers and particles with (A E) LP9/TERT 1 human mesothelial cells and (F) IOSE ovarian epithelial cells after 24 hours of exposure to (B, E, F) high and (C, D) low concentrations of particulates. (G) Field emission scanning electron microscopy (FESEM) and (H) transmission electron microscopy (TEM) show structure of nonfibrous talc. (A) Morphology of unexposed near confluent LP9/TERT 1 cells. (B) Membrane blebbing and piling up of cells in response to crocidolite asbestos (arrows). (C) Nonfibrous talc and (D) fine TiO₂ (arrows) on cell surface. (E) Single and small clumps of glass beads on plasma membrane. (F) Interaction of asbestos fibers (arrows) with IOSE cells that exhibit an exudate and membrane ruffling in response to fibers. Bars 10 μm. (G) FESEM and (H) TEM showing morphology of platy talc bulk material. Bars 2 μm.

Transfection of LP9 Cells with siRNA

On Target plus Non targeting siRNA #1 (scrambled control), and On Target plus SMART pool human *ATF3* siRNA (100 nM; Dharmacon, Lafayette, CO) were transfected into LP9 cells at near confluence using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), following the manufacturer's protocol. The efficiency of *ATF3* knockdown was determined by QRT PCR after 48 and 72 hours.

Bio Plex Analysis of Cytokine and Chemokine Concentrations in Medium of LP9/TERT 1 Cells

To quantify cytokine and chemokine levels in conditioned medium of cells transfected with siATF3 or scrambled control and exposed to

asbestos for 24 hours, a multiplex suspension protein array was performed using the Bio Plex protein array system as described previously (17) and a Human Cytokine 27 plex panel (Bio Rad, Hercules, CA). Three biological replicates were used for each treatment group.

Statistical Analysis

Data from QRT PCR and cell viability assays were evaluated by ANOVA using the Student Neuman Keul's procedure for adjustment of multiple pairwise comparisons between treatment groups or using the nonparametric Kruskal Wallis and Mann Whitney tests. Differences with *P* values ≤ 0.05 were considered statistically significant.

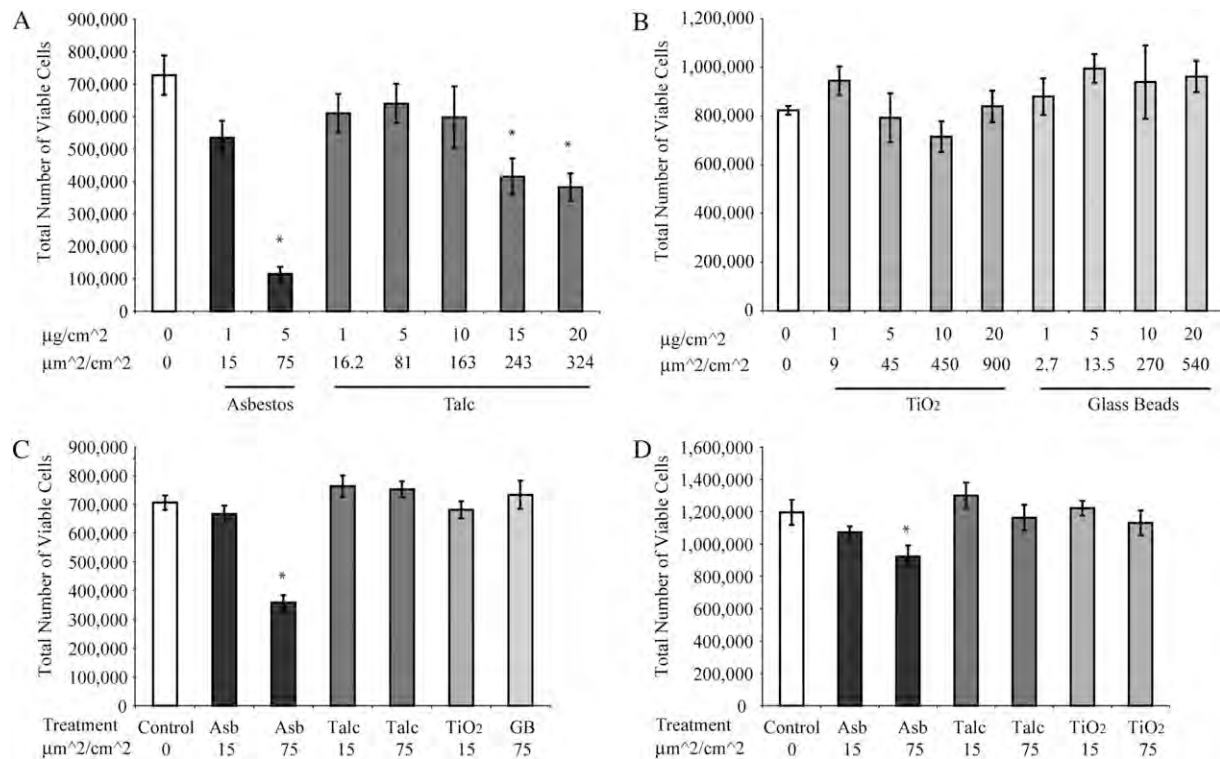


Figure 2. Cell viability after 24 hours of exposure to asbestos fibers and particles in (A) LP9/TERT 1 and (D) IOSE (D). Mean \pm SE of 1 (A, B) or 3 (C, D) individual experiments where $n = 3$ per group per experiment. * $P \leq 0.05$ compared with untreated (0) groups.

RESULTS

Characterization of Particulate Preparations

Table 1 shows the major chemical formulas of crocidolite asbestos fibers (defined as having a greater than 3:1 length to width ratio) and particle samples used in experiments, although trace amounts of other elements occur in the NIEHS asbestos standards (15). In addition, we examined the morphology and cellular interactions of asbestos fibers, talc, and other particles using SEM (Figure 1). These studies revealed that only high ($75 \mu\text{m}^2/\text{cm}^2$) surface area concentrations of asbestos caused membrane blebbing and other toxic manifestations in cells (Figures 1B and 1F). In contrast, particles of nonfibrous talc (Figure 1C), fine TiO₂ (Figure 1D), and glass beads (Figure 1E) were nontoxic. Both asbestos fibers and particles were observed on the cell surface and were encompassed by cells. Nonfibrous talc occurred in platy particles that were uniform in appearance as viewed by FESEM (Figure 1G) and TEM (Figure 1H).

Asbestos Fibers at High Concentrations Are Toxic to LP9/TERT 1 Human Mesothelial Cells and Less So to Ovarian Epithelial Cells in Contrast to Particle Preparations

Figure 2 shows the results of trypan blue exclusion tests in LP9/TERT 1 and IOSE cells. In LP9/TERT 1 cells (Figures 2A, 2C), asbestos at high surface area concentrations ($75 \mu\text{m}^2/\text{cm}^2$) caused significant decreases (50–80%) in cell viability that were more striking than those observed in IOSE cells (Figure 2D). Nonfibrous talc at $75 \mu\text{m}^2/\text{cm}^2$ was nontoxic, and significant increases in toxicity were only achieved with addition of talc at ≥ 3 fold higher concentrations in LP9/TERT 1 cells (Figure 2A), but not in IOSE cells (data not shown). Neither TiO₂ nor glass beads were significantly toxic to either cell type over a range of concentrations (Figure 2B).

Asbestos Fibers, but Not Particle Preparations, Cause Dose- and Time Related Changes in Gene Expression in Human LP9 Mesothelial Cells

Figure 3 shows a summary of significantly increased or decreased (> 2 fold compared with untreated controls) gene expression by asbestos (Figures 3A–3C) and nonfibrous talc (Figure 3D) in LP9/TERT 1 cells as well as the classification of genes by ontology. These studies revealed that gene expression changes by low concentrations of asbestos were less (29 increases) than at high concentrations (236 alterations including decreases) at 8 hours. Moreover, numbers of significant mRNA level alterations (205) at low concentrations of asbestos increased over time. In contrast, fewer numbers (30) of gene expression increases were observed at high concentrations of talc at 8 hours compared with identical surface areas of asbestos (236 changes), and no decreases in gene expression were observed. No significant alterations in gene expression were observed with low concentrations of talc at 24 hours or with TiO₂ or glass beads at either concentration or time point (data not shown). The major genes affected by asbestos or talc in LP9/TERT 1 cells are listed in Tables 2–4. This information reveals that the fold increases in common genes expressed by asbestos treated cells increase in a dose related fashion at 8 hours. Although dose responses were observed with talc at 8 hours, the numbers of significant gene increases as well as fold increases were less than that observed with asbestos and decreased over time. Since mRNA expression of *ATF3* and *IL8* were increased by either asbestos or talc in LP9/TERT 1 cells, the increased expression of these genes was verified by QRT-PCR in mineral exposed cells as compared with untreated control cells (Figure 4).

In NYU474 cells, QRT-PCR was used to validate that eight asbestos induced genes in LP9 cells were up-regulated in

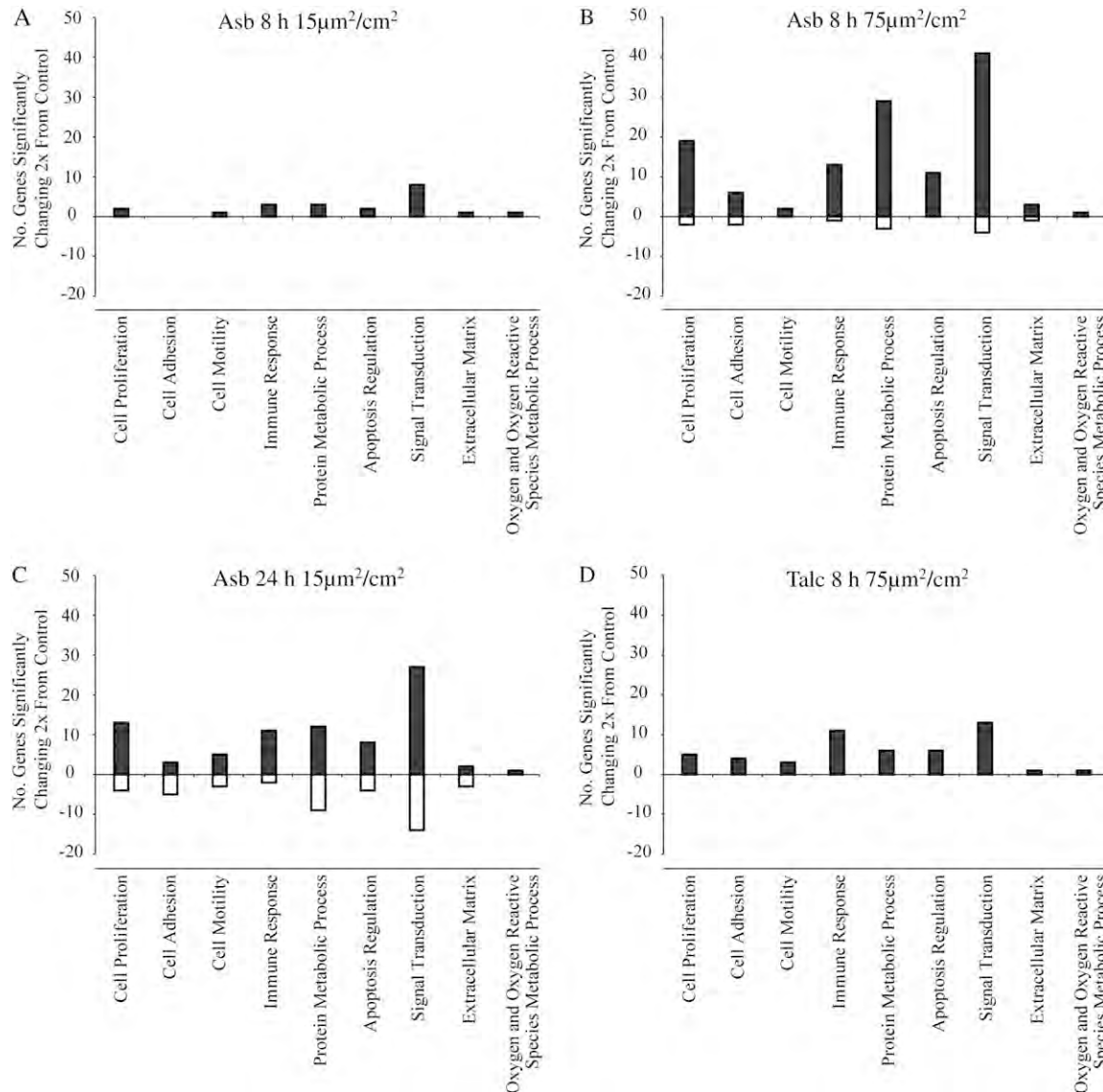


Figure 3. Numbers of changes ($P \leq 0.05$) in gene expression and classification by ontology in LP9/TERT 1 cells after exposure to (A) (C) crocidolite asbestos or (D) nonfibrous talc.

normal human mesothelial cells (*ATF3*, *PTGS2* or *COX2*, *FOSB*, *IL8*, *NR4A2*, and *TFPI2*). Results showed that mRNA levels of six of the eight genes evaluated were increased in a dose responsive fashion after exposure to asbestos for 24 hours (Figure 5).

IOSE Ovarian Epithelial Cells Exhibit Few Gene Expression Changes in Response to Asbestos

In contrast to LP9/TERT 1 and NYU474 mesothelial cells, IOSE cells showed no significant gene up regulation or down regulation in response to lower concentrations of asbestos at 8 or 24 hours (data not shown). At high concentrations of asbestos at 8 hours, mRNA levels of only two genes (*NR4A2* and *CXCL2* or *MIP2*) were increased in comparison to untreated IOSE cells (Table 4). At 24 hours, high concentrations of asbestos caused less than 4 fold increases in expression of only 16 genes, and decreased expression of 1 gene, *Profilin 1* (data not shown). No significant mRNA changes were observed with nonfibrous talc, fine TiO_2 or glass beads at either time point.

Inhibition of *ATF3* by siRNA Alters Asbestos Induced Cytokines in LP9/TERT 1 Cells

Since *ATF3* was a common gene up regulated by asbestos in mesothelial cells its functional role in cytokine production in LP9 cells was evaluated. As shown in Figure 6A, *ATF3* was successfully inhibited in LP9/TERT 1 cells using siATF3 as described in MATERIALS AND METHODS. Cells transfected with control siRNA or siATF3 were then exposed to asbestos (75 $\mu\text{m}^2/\text{cm}^2$ $n = 3$) for 24 hours, and medium was collected and analyzed for cytokines and growth factors using Bio Plex analyses. Inhibition of *ATF3* altered levels of asbestos induced inflammatory cytokines (IL 1 β , IL 13, G CSF) and the growth factor (PGDF BB) in LP9/TERT 1 cells (Figure 6B). Trends in diminishing levels of VEGF were also observed, although not statistically significant.

DISCUSSION

Gene expression analysis has been used for the classification of soluble toxicants in rodent and human cells *in vitro*. Models of

TABLE 2. TOP 10 GENES AFFECTED BY CROCIDOLITE ASBESTOS AT 8 AND 24 H IN LP9/TERT 1 HUMAN MESOTHELIAL CELLS

Concentration	Low (15 μm ² /cm ²)		High (75 μm ² /cm ²)
Time	8 h	24 h	8 h
Fold Change			
Up-regulated			
Activating transcription factor 3 (ATF3)	9	9	27
Prostaglandin-endoperoxide synthase 2 (PTGS2)	7	8	16
Superoxide Dismutase 2 (SOD2)	6	6	2
Chemokine (C-X-C motif) ligand 3 (CXCL3)	4	NC	16
FBJ murine osteosarcoma viral oncogene homolog B (FOSB)	4	NC	NC
Tissue factor pathway inhibitor 2 (TFPI2)	4	14	11
Pyruvate dehydrogenase kinase, isozyme 4 (PDK4)	3	9	15
Chemokine (C-X-C motif) ligand 2 (CXCL2)	3	NC	NC
Angiopoietin-like 4 (ANGPLT4)	3	NC	NC
Kruppel-like factor 4 (gut) (KLF4)	3	NC	NC
Interleukin 8 C-terminal variant, 211506 s t (IL8)	NC	8	12
Interleukin 1 receptor-like 1 (IL1R1)	NC	6	11
Nuclear receptor subfamily 4 (NR4A2)	NC	NC	11
Solute carrier family 7 (SLC7A2)	NC	6	10
Pleckstrin homology-like domain (PHLDA1)	NC	7	NC
Interleukin 8 (IL8)	NC	6	NC
Down-regulated			
Inhibitor of DNA binding 3 (ID3)	NC	NC	5
Inhibitor of DNA binding 1 (ID1)	NC	NC	3
Cytochrome P450, family 24 (CYP24A1)	NC	NC	3
Basic helix-loop-helix domain (BHLHB3)	NC	NC	3
SMAD family member 6 (SMAD6)	NC	NC	3
S-phase kinase associated protein 2 (SKP2)	NC	NC	3
Cadherin 10, type 2 (CDH10)	NC	NC	3
START domain containing 5 (STARD5)	NC	NC	3
211042 x at	NC	NC	2
Interferon-induced protein with tetratricopeptide (IFIT1)	NC	NC	2
Oxytocin receptor (OXTR)	NC	6	NC
Transcribed locus	NC	5	NC
Chromosome 5 open reading frame (C5orf13)	NC	5	NC
Cytochrome P450, family 24 (CYP24A1)	NC	4	NC
Chromosome 21 open reading frame (C21orf7)	NC	3	NC
KIAA1199	NC	3	NC
Methyltransferase like 7A (METTL7A)	NC	3	NC
PDZ domain containing RING finger 3 (PDZRN3)	NC	3	NC
Periplakin (PPL)	NC	3	NC
Phospholipase-C-like 1 (PLCL1)	NC	3	NC

Definition of abbreviation: NC, no significant ($P \leq 0.05$) change > 2-fold from control.

transcript profiling for discrimination of toxic and nontoxic compounds in liver and other organs have also been developed in rodents (18), confirming the hypothesis that predictive modeling for classification of toxic agents and carcinogens is feasible. Here we used toxicogenomic approaches in human mesothelial cells, a cell type exquisitely sensitive to asbestos (19) and human contact inhibited ovarian epithelial cells, a cell type not linked to carcinogenesis by asbestos, to determine whether the magnitude of altered gene expression by insoluble particulates correlated with their toxicity to cells and documented pathogenicity in humans. Although a recent study has examined gene expression profiles comparatively in crocidolite asbestos exposed human lung adenocarcinoma (A549) and SV40 immortalized bronchial (BEAS 2B) or pleural mesothelial cell lines (MET5A) by cluster analysis (20), our studies are the first to examine gene expression changes by asbestos in comparison to other well characterized particles in a human cell line that exhibits features of normal mesothelial cells (5). Although strict comparisons between cell types are not justified because SV40 Tag was used to immortalize the IOSE ovarian epithelial cell line (6), and SV40 infection is known to decrease sensitivity of human mesothelial cell lines to toxicity by asbestos

(21), our studies suggest that the increased numbers of gene expression alterations observed in LP9/TERT 1 human mesothelial cells reflect elevated sensitivity of this cell type to asbestos. NYU474 human mesothelial cells were more resistant than LP9/TERT 1 cells to asbestos toxicity, permitting us to perform QRT PCR studies at both concentrations of asbestos at 24 hours. These results confirmed common dose related patterns of gene expression in mesothelial cells versus ovarian epithelial (IOSE) cells.

It is generally recognized that geometry and length and width (i.e., aspect ratio) of durable fibers such as amphibole asbestos types (crocidolite, amosite) are important properties determining toxicity, transforming potential, and carcinogenicity in rodents and humans (13, 22, 23). Since talc can occur in various geometries (nonfibrous and fibrous) and can be contaminated with other minerals, including amphiboles, in some mining deposits (reviewed in Ref. 24), we used a well characterized, nonfibrous talc sample here to allow evaluation of a particle not causing mesotheliomas or pleural sarcomas in rodents (23). Moreover, nonfibrous talc is regarded as noncarcinogenic in humans (25). Since talc is a magnesium silicate, and Mg^{2+} may interact with negatively charged molecules on the cell surface to

TABLE 3. GENES UP REGULATED BY NONFIBROUS TALC IN LP9/TERT 1 HUMAN MESOTHELIAL CELLS

Gene	Fold Increase
8 h Low (15 $\mu\text{m}^2/\text{cm}^2$)	
Activating transcription factor 3 (ATF3)	3
8 h High (75 $\mu\text{m}^2/\text{cm}^2$)	
Activating transcription factor 3 (ATF3)	13
Inhibin, beta A (INHBA)	9
Chemokine (C-X-C motif) ligand 3 (CXCL3)	7
Superoxide dismutase 2 (SOD2)	7
Interleukin 8 C-terminal variant, 211506 s t (IL8)	6
Prostaglandin-endoperoxide synthase 2 (PTGS2)	5
Interleukin 8 (IL8)	5
FBJ murine osteosarcoma viral oncogene homolog B (FOSB)	5
Tumor necrosis factor alpha-induced protein 6 (TNFAIP6)	4
Tissue factor pathway inhibitor 2 (TFPI2)	4
Chemokine (C-X-C motif) ligand 2 (CXCL2)	3
Intercellular adhesion molecule 4 (ICAM4)	3
ChaC, cation transport regulator homolog 1 (ChaC 1)	3
Nuclear receptor subfamily 4, group A, member 3 (NR4A3)	3
Pleckstrin homology-like domain, family A, member 1 (PHLDA1)	3
Interleukin 6 (IL-6)	3
Phorbol -12-myristate-13-acetate-induced protein 1 (PMA1P1)	3
Oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1)	3
Chemokine (C-C motif) ligand 20 (CCL20)	3
v-maf musculoaponeurotic fibrosarcoma oncogene homolog F	3
Interleukin 1, alpha (IL-1 α)	2
Tumor necrosis factor- α induced protein 3 (TNFAIP3)	2
Interleukin 1 receptor-like 1 (IL1RL1)	2
Angiopoietin-like 4 (ANGPLT4)	2
Kruppel-like factor 4 (KLF4)	2
GTP binding protein overexpressed in skeletal muscle (GEM)	2
Pentraxin-related gene, rapidly induced by IL-1 beta (PTX3)	2
Interleukin 1 beta (IL-1 β)	2
HSPB (heat shock 27 kD) associated protein 1 (HSPBAP1)	2
Kynureninase (KYNUN)	2

disturb cell homeostasis (reviewed in Ref. 26), this may explain the few mRNA expression increases that were observed initially with talc at 8 hours. However, these changes were not observed at 24 hours, suggesting that human mesothelial cells adapt to or undergo repair after exposure to this mineral.

Our gene profiling data here and in inhalation studies using chrysotile asbestos (14) also support the concept that fine TiO_2 is nontoxic and nonpathogenic to mesothelial or other cell

TABLE 4: GENES UPREGULATED BY CROCIDOLITE ASBESTOS IN IOSE HUMAN OVARIAN CELLS

Gene	Fold increase
8 h High (75 $\mu\text{m}^2/\text{cm}^2$)	
Nuclear receptor subfamily 4 (NR4A2)	4
Chemokine (C-X-C motif) ligand 2 (MIP2)	2
24 h High (75 $\mu\text{m}^2/\text{cm}^2$)	
Nuclear receptor subfamily 4 (NR4A2)	4
DNA-damage-inducible transcript 3 (DDIT3)	3
Stromal cell-derived factor 2-like 1 (SDF2L1)	3
Heat shock 70 kD protein 1A (HSPA1A)	3
Dnaj (Hsp40) homolog, subfamily C (DNAJC3)	2
Paraspeckle component 1	2
Heat shock 70 kD protein 1B (HSPA1B)	2
Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member (HERPUD1)	2
Serum/glucocorticoid regulated kinase family, member 3 (SKG3)	2
Dnaj (Hsp40) homolog, subfamily B, member 9 (DNAJB9)	2
Arginine-rich, mutated in early stage tumors (ARMET)	2
Syntaxin 1A (brain) (STX1A)	2
Heat shock 70 kD protein 5 (HSPA5)	2
ADAM metalloproteinase with thrombospondin type 1 motif	2
Heat shock protein 90kDa beta (Grp94), member 1 (HSP90B1)	2

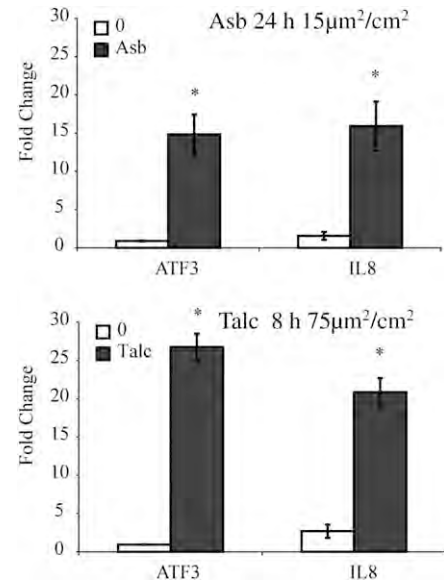


Figure 4. QRT PCR confirms significant increases in *ATF3* and *IL8* expression by crocidolite asbestos at low concentrations and non fibrous talc at high concentrations in LP9/TERT 1 mesothelial cells. * $P < 0.05$ as compared to untreated (0) groups.

types. Likewise, in the rat, inhalation of fine TiO_2 (defined as particles $> 0.1 \mu\text{m}$ in diameter), in contrast to ultrafine (particles $< 0.1 \mu\text{m}$ in diameter) does not give rise to predictive markers of toxicity, inflammation, pulmonary fibrosis, or oxidative stress, as indicated by elevated levels of Mn containing superoxide dismutase (*SOD2*) in cells from bronchopulmonary lavage (27). The increased reactivity and toxicity of ultrafine particles as compared with larger fine or coarse particles have also been confirmed in a number of *in vitro* and *in vivo* experiments and is often attributed to their increased surface area and/or ability to penetrate lung cells.

Our studies reveal a number of novel genes induced by asbestos in LP9/TERT 1 cells. As previously described in a lung epithelial cell line (C10) or mouse lungs after inhalation of crocidolite asbestos (28), increases in expression of the early response gene, *FOSB*, that encodes a dimer of the activator protein 1 transcription factor, were seen. Increases in expression of several other genes linked to cell signaling proteins and transcription factor activation were observed in asbestos exposed cells, including *NR4A2* and *PDK4*. A novel gene up regulated at all time points and concentrations of asbestos or talc in human mesothelial cells was activating transcription factor 3 (*ATF3*), a member of the cAMP responsive element binding (CREB) transcription factor family that encodes two different isoforms leading to repression or activation of genes. Silencing of *ATF3* in the present study by siRNA significantly altered expression of a number of asbestos induced inflammatory cytokines and growth factors documented in malignant mesotheliomas (29, 30). In support of our results here, other studies using *ATF3* deficient mice and *in vitro* approaches have shown that *ATF3* is a negative regulator of pulmonary inflammation, eosinophilia, and airway responsiveness (31). Moreover, *ATF3* also negatively regulates IL 6 gene transcription in an NF κB model of up regulation using melanoma cells (32). In addition, trends in production of VEGF, a known important angiogenic peptide and independent prognostic factor in human mesotheliomas (33), were observed. We have recently shown that an extracellular signal related

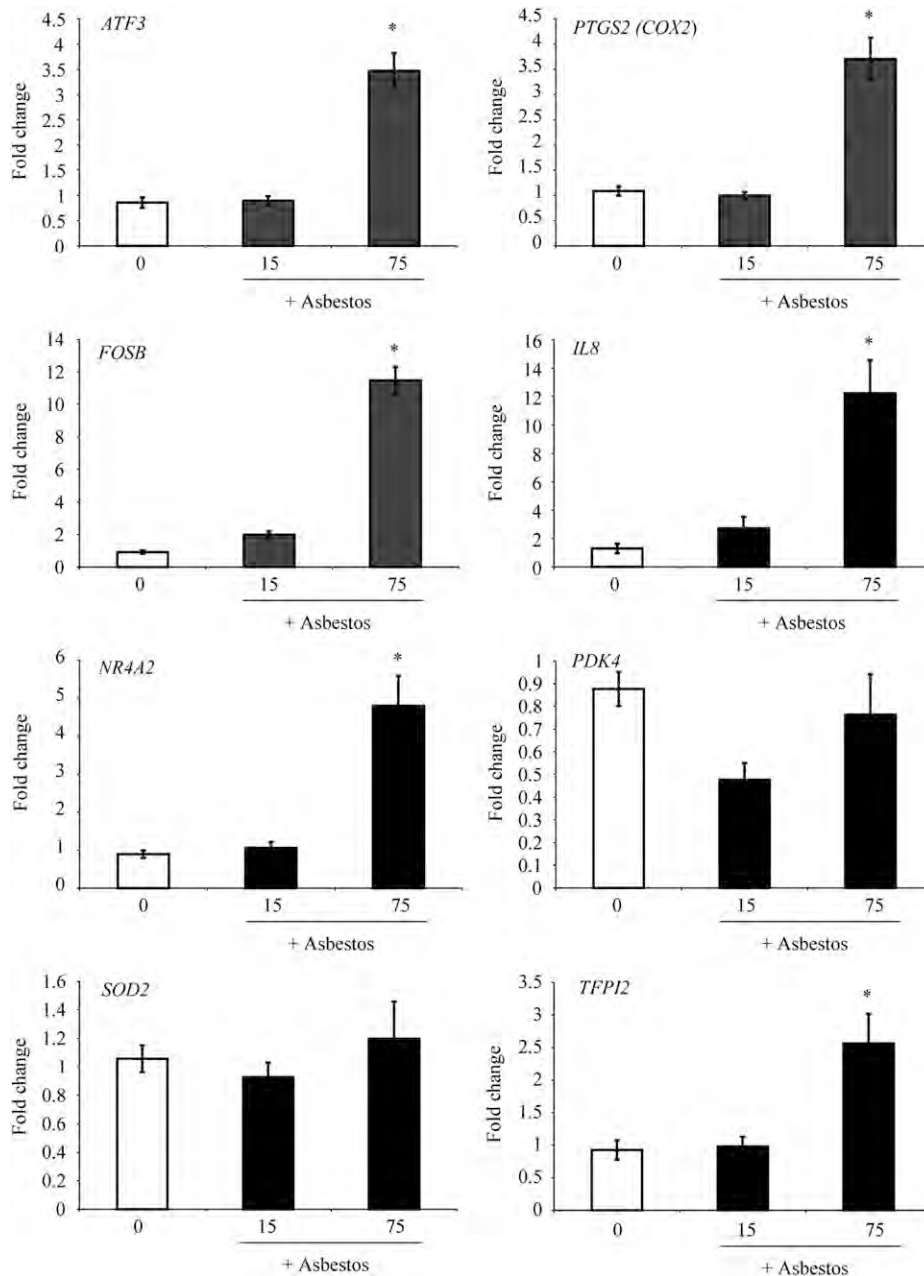


Figure 5. QRT PCR confirms that human primary pleural mesothelial cells (NYU474) show similar patterns of asbestos induced gene expression when compared with LP9/TERT 1 mesothelial cells. NYU474 cells were exposed to crocidolite asbestos (15 or 75 $\mu\text{m}^2/\text{cm}^2$) for 24 hours and cDNA was used for QRT PCR. * $P \leq 0.05$ as compared with untreated cells (0).

CREB pathway in C10 lung epithelial cells modulates apoptosis after asbestos exposure (34), and recent studies are focusing on the effects of silencing *CREB* or *ATF3* on other functional and phenotypic changes in human mesothelial and mesothelioma cells (A. Shukla and colleagues, unpublished data).

Several other genes up regulated by talc at 8 hours or affected by asbestos at both 8 and 24 hours may be important in repair from mineral induced responses. For example, *SOD2*, (Mn containing superoxide dismutase) is an antioxidant protein occurring in the mitochondria, a target cell organ of asbestos induced apoptosis (35). *PTGS2* (prostaglandin endoperoxide synthase or cyclooxygenase) is a key enzyme in prostenoid biosynthesis associated with modulation of mitogenesis and inflammation. More recently, this pathway has been explored after interaction of ultrafine particles with alveolar macrophages (9). *ANG PTL4* (angiopoietin 4) encodes a serum hormone directly involved in regulating glucose homeostasis and lipid metabolism and is an apoptosis survival factor for vascular endothelial cells. The up regulation of angio-

poietin 4 is also thought to play a role in inhibition of tumor cell motility and metastasis. *KLF4* (Kruppel like factor 4) is a negative regulator of cell proliferation and can be a positive or negative modulator of DNA transcription.

Increased expression of genes encoding different cytokines/chemokines (i.e., *IL8*) and their receptors or ligands (e.g., IL 8 C terminal variant, *IL1RI*, *CXCL2* or *MIP2*, *CXCL3*, and *TFPI2*) by asbestos or talc suggests that the mesothelial cell also may play a role in chemotaxis, inflammation, and blood coagulation. A number of gene expression changes by asbestos also support the hypothesis that this fibrous mineral affects calcium dependent processes including related protein kinase cascades, cell adhesion, and protein/lipid metabolism (Table 2). Although numbers of changes were more modest in IOSE cells, with the exception of *NR4A2* and *CXCL2*, a unique subset of genes was induced by asbestos in this cell type (Table 4).

Results of work here suggest that transcriptional profiling can be used to reveal molecular events by mineral dusts that are

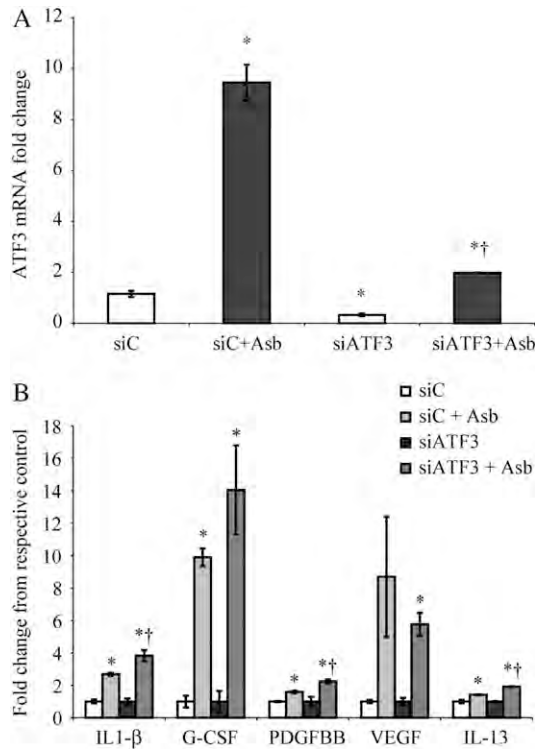


Figure 6. ATF3 inhibition using siRNA approaches alters asbestos induced production of inflammatory cytokines and growth factors. (A) LP9/TERT 1 cells transfected with siATF3 show significant inhibition of ATF3 mRNA levels (untreated control [siC] versus siATF3 and asbestos treated [siC Asb versus siATF3 Asb] groups). * $P \leq 0.05$ as compared with siC; † $P \leq 0.05$ as compared with siC Asb group. (B) siATF3 altered asbestos induced cytokine levels as detected in medium at 24 hours using Bio Plex analyses. * $P \leq 0.05$ as compared with control groups (siC and siATF3), respectively; † $P \leq 0.05$ as compared with asbestos exposed scrambled control group (siC).

predictive of their pathogenicity in mesothelioma. Moreover, they reveal early and novel gene responses, including calcium dependent transcription factors and antioxidant enzymes that may be pursued for their functional significance using RNA silencing or other approaches.

Conflict of Interest Statement: B.T.M. received support by EUROTALC and The Industrial Minerals Association (IMA) (11/1/05–10/31/06) for \$90,000 for research. None of the other authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors thank the Vermont Cancer Center DNA Analysis Facility for performing oligonucleotide microarray and real-time quantitative PCR, and Gary Tomiano (Minteq International, Inc./Specialty Minerals, Inc., Easton, PA) for talc characterization.

References

- Mossman BT, Gee JB. Asbestos related diseases. *N Engl J Med* 1989; 320:1721–1730.
- Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med* 1998;157:1666–1680.
- Robinson BW, Lake RA. Advances in malignant mesothelioma. *N Engl J Med* 2005;353:1591–1603.
- Mossman BT, Bignon J, Corn M, Seaton A, Gee JB. Asbestos: scientific developments and implications for public policy. *Science* 1990;247: 294–301.
- Dickson MA, Hahn WC, Ino Y, Ronfard V, Wu JY, Weinberg RA, Louis DN, Li FP, Rheinwald JG. Human keratinocytes that express hTERT and also bypass a p16(INK4a) enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol Cell Biol* 2000;20:1436–1447.

- Choi JH, Choi KC, Auersperg N, Leung PC. Overexpression of follicle stimulating hormone receptor activates oncogenic pathways in pre neoplastic ovarian surface epithelial cells. *J Clin Endocrinol Metab* 2004;89:5508–5516.
- Merritt MA, Green AC, Nagle CM, Webb PM. Talcum powder, chronic pelvic inflammation and NSAIDs in relation to risk of epithelial ovarian cancer. *Int J Cancer* 2008;122:170–176.
- Mossman BT, Shukla A, Fukagawa NK. Highlight Commentary on “Oxidative stress and lipid mediators induced in alveolar macrophages by ultrafine particles”. *Free Radic Biol Med* 2007;43:504–505.
- Beck Speier I, Dayal N, Karg E, Maier KL, Schumann G, Schulz H, Semmler M, Takenaka S, Stettmaier K, Bors W, et al. Oxidative stress and lipid mediators induced in alveolar macrophages by ultrafine particles. *Free Radic Biol Med* 2005;38:1080–1092.
- Oberdorster G, Ferin J, Gelein R, Soderholm SC, Finkelstein J. Role of the alveolar macrophage in lung injury: studies with ultrafine particles. *Environ Health Perspect* 1992;97:193–199.
- Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. Size dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicol Appl Pharmacol* 2001;175:191–199.
- Donaldson K, Tran CL. Inflammation caused by particles and fibers. *Inhal Toxicol* 2002;14:5–27.
- Health Effects Institute. Asbestos Research. Asbestos in public and commercial buildings: a literature review and synthesis of current knowledge. Cambridge, MA: The Health Effects Institute; 1991.
- Sabo Attwood T, Ramos Nino M, Bond J, Butnor KJ, Heintz N, Gruber AD, Steele C, Taatjes DJ, Vacek P, Mossman BT. Gene expression profiles reveal increased mClca3 (Gob5) expression and mucin production in a murine model of asbestos induced fibrogenesis. *Am J Pathol* 2005;167:1243–1256.
- Campbell WJ, Huggins CW, Wylie AG. Chemical and physical characterization of amosite, chrysotile, crocidolite, and nonfibrous tremolite for oral ingestion studies. Washington, DC: National Institute of Environmental Health Sciences; 1980. No. 8542.
- Blumen SR, Cheng K, Ramos Nino ME, Taatjes DJ, Weiss DJ, Landry CC, Mossman BT. Unique uptake of acid prepared mesoporous spheres by lung epithelial and mesothelioma cells. *Am J Respir Cell Mol Biol* 2007;36:333–342.
- Shukla A, Lounsbury KM, Barrett TF, Gell J, Rincon M, Butnor KJ, Taatjes DJ, Davis GS, Vacek P, Nakayama KI, et al. Asbestos induced peribronchiolar cell proliferation and cytokine production are attenuated in lungs of protein kinase C delta knockout mice. *Am J Pathol* 2007;170:140–151.
- Steiner G, Suter L, Boess F, Gasser R, de Vera MC, Albertini S, Ruepp S. Discriminating different classes of toxicants by transcript profiling. *Environ Health Perspect* 2004;112:1236–1248.
- Lechner JF, Tokiwa T, LaVeck M, Benedict WF, Banks Schlegel S, Yeager H Jr, Banerjee A, Harris CC. Asbestos associated chromosomal changes in human mesothelial cells. *Proc Natl Acad Sci USA* 1985;82:3884–3888.
- Nymark P, Lindholm PM, Korpela MV, Lahti L, Ruosaari S, Kaski S, Hollmen J, Anttila S, Kinnula VL, Knuutila S. Gene expression profiles in asbestos exposed epithelial and mesothelial lung cell lines. *BMC Genomics* 2007;8:62.
- Cacciotti P, Barbone D, Porta C, Altomare DA, Testa JR, Mutti L, Gaudino G. SV40 dependent AKT activity drives mesothelial cell transformation after asbestos exposure. *Cancer Res* 2005;65:5256–5262.
- Davis JM, Addison J, Bolton RE, Donaldson K, Jones AD, Smith T. The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. *Br J Exp Pathol* 1986;67:415–430.
- Stanton MF, Layard M, Tegeris A, Miller E, May M, Morgan E, Smith A. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. *J Natl Cancer Inst* 1981;67:965–975.
- Guthrie GD Jr, Mossman BT. Health effects of mineral dusts. Washington, DC: Mineralogical Society of America; 1993.
- IARC. Silica and some silicates. *IARC Monogr Eval Carcinog Risk Chem Hum* 1987;42:185.
- Mossman B, Light W, Wei E. Asbestos: mechanisms of toxicity and carcinogenicity in the respiratory tract. *Annu Rev Pharmacol Toxicol* 1983;23:595–615.
- Janssen YM, Heintz NH, Mossman BT. Induction of c fos and c jun proto oncogene expression by asbestos is ameliorated by N acetyl L cysteine in mesothelial cells. *Cancer Res* 1995;55:2085–2089.

28. Ramos Nino ME, Heintz N, Scappoli L, Martinelli M, Land S, Nowak N, Haegens A, Manning B, Manning N, MacPherson M, et al. Gene profiling and kinase screening in asbestos exposed epithelial cells and lungs. *Am J Respir Cell Mol Biol* 2003;29:S51 S58.
29. Yoshimoto A, Kasahara K, Saito K, Fujimura M, Nakao S. Granulocyte colony stimulating factor producing malignant pleural mesothelioma with the expression of other cytokines. *Int J Clin Oncol* 2005;10:58 62.
30. Vogelzang NJ, Herndon JE II, Miller A, Strauss G, Clamon G, Stewart FM, Aisner J, Lyss A, Cooper MR, Suzuki Y, et al. High dose paclitaxel plus G CSF for malignant mesothelioma: CALGB phase II study 9234. *Ann Oncol* 1999;10:597 600.
31. Gilchrist M, Henderson WR Jr, Clark AE, Simmons RM, Ye X, Smith KD, Aderem A. Activating transcription factor 3 is a negative regulator of allergic pulmonary inflammation. *J Exp Med* 2008;205:2349 2357.
32. Karst AM, Gao K, Nelson CC, Li G. Nuclear factor kappa B subunit p50 promotes melanoma angiogenesis by upregulating interleukin 6 expression. *Int J Cancer* 2009;124:494 501.
33. Demirag F, Unsal E, Yilmaz A, Caglar A. Prognostic significance of vascular endothelial growth factor, tumor necrosis, and mitotic activity index in malignant pleural mesothelioma. *Chest* 2005;128: 3382 3387.
34. Barlow CA, Barrett TF, Shukla A, Mossman BT, Lounsbury KM. Asbestos mediated CREB phosphorylation is regulated by protein kinase A and extracellular signal regulated kinases 1/2. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L1361 L1369.
35. Shukla A, Stern M, Lounsbury KM, Flanders T, Mossman BT. Asbestos induced apoptosis is protein kinase C delta dependent. *Am J Respir Cell Mol Biol* 2003;29:198 205.

Exhibit 94

Pycnogenol® reduces Talc-induced Neoplastic Transformation in Human Ovarian Cell Cultures

Amber R. Buz'Zard* and Benjamin H. S. Lau

Department of Biochemistry and Microbiology, School of Medicine, Loma Linda University, Loma Linda, CA 92350, USA

Talc and poor diet have been suggested to increase the risk of developing ovarian cancer; which can be reduced by a diet rich in fruit and vegetables. Talc is ubiquitous despite concern about its safety, role as a possible carcinogen and known ability to cause irritation and inflammation. It was recently shown that Pycnogenol® (Pyc; a proprietary mixture of water-soluble bioflavonoids extracted from French maritime pine bark) was selectively toxic to established malignant ovarian germ cells. This study investigated talc-induced carcinogenesis and Pyc-induced chemoprevention. Normal human epithelial and granulosa ovarian cell lines and polymorphonuclear neutrophils (PMN) were treated with talc, or pretreated with Pyc then talc. Cell viability, reactive oxygen species (ROS) generation and neoplastic transformation by soft agar assay were measured. Talc increased proliferation, induced neoplastic transformation and increased ROS generation time-dependently in the ovarian cells and dose-dependently in the PMN. Pretreatment with Pyc inhibited the talc-induced increase in proliferation, decreased the number of transformed colonies and decreased the ROS generation in the ovarian cells. The data suggest that talc may contribute to ovarian neoplastic transformation and Pyc reduced the talc-induced transformation. Taken together, Pyc may prove to be a potent chemopreventative agent against ovarian carcinogenesis. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: ovarian cancer; talc; Pycnogenol®; human neutrophils.

INTRODUCTION

Ovarian cancer is the sixth most commonly occurring cancer and ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system. Epidemiological studies have suggested that diet, talc, industrial pollutants, smoking, asbestos and infectious agents may increase the risk of developing ovarian cancer (American Cancer Society, 2000) and may do so by causing localized inflammation (Ness and Cottreau, 1999). Specifically, talc exposure has been cited as a risk factor because of its similarity to asbestos (Cramer *et al.*, 1999).

Talc is a layered magnesium silicate [$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$]. It is used in cosmetics (as the primary ingredient in talcum powder), pharmaceuticals (as an excipient in tablets) and in many other industrial applications (Bremmell and Addai-Mensah, 2005). Talc is used medically to induce pleurodesis because of its known ability to cause irritation and inflammation (Holthouse and Chleboun, 2001). Animal studies showed a systemic migration of talc particles to various organs despite route of entry (Henderson *et al.*, 1986; Werebe *et al.*, 1999). Exposure of rat ovaries to talc leads to cyst formation (Hamilton *et al.*, 1984). Talc was also shown to cause superoxide anion generation and release from murine macrophages (Van Dyke *et al.*, 2003). Thus controversy

continues to surround the topic of talc, its safety (Janssen, 2004) and its role as a possible carcinogen (Cramer *et al.*, 1999; Wong *et al.*, 1999).

Lifestyle factors are important in the etiology of ovarian cancer and current evidence suggests the risk can be reduced by eating a diet rich in fruit and vegetables, among other lifestyle choices (Hanna and Adams, 2006). For the past 20 years, researchers have proposed that nutritional factors play one of the most important roles in the etiology of human cancer. It is estimated that 35% (range 10–70%) of all cancers are diet related and that consumption of certain fruits and vegetables is inversely associated with the incidence of specific forms of cancer. Past research has indicated that a large number of bioactive components, which proved to be protective on different stages of cancer formation, have been identified in nutrients that are of plant origin (Knasmüller and Verhagen, 2002).

Pycnogenol® (Pyc) is a proprietary mixture of water-soluble bioflavonoids extracted from the bark of French maritime pine (*Pinus maritima* Aiton; currently known as *Pinus pinaster* Aiton). The main constituents of Pyc are phenolic compounds, broadly divided into monomers (catechin, epicatechin and taxifolin) and condensed flavonoids (classified as procyanidins and proanthocyanidins). Pyc is known to possess potent antioxidant activity, it not only scavenges the free radicals but it also enhances the endogenous antioxidant systems (Nelson *et al.*, 1998; Wei *et al.*, 1997). Pyc has also been shown to selectively induce apoptosis in breast cancer cells (Huyhn and Teel, 2000) and induce differentiation and apoptosis in human promyeloid leukemia cells (Huang *et al.*, 2005). It was previously

* Correspondence to: Dr Amber R. Buz'Zard, Department of Biochemistry and Microbiology, School of Medicine, Loma Linda University, Loma Linda, CA 92350, USA.

E-mail: abuzzard03b@llu.edu

Contract/grant sponsor: Horphag Research, Geneva, Switzerland.

shown that Pyc selectively induced cell death in established malignant ovarian germ cells *in vitro* (Buz'Zard and Lau, 2004). This study now reports that Pyc prevents talc-induced neoplastic transformation of normal ovarian cells, *in vitro*.

MATERIALS AND METHODS

Reagents and chemicals. Pycnogenol® was supplied by Horphag Research (Geneva, Switzerland). Talc, crystal violet, Giemsa stain, RPMI-1640 medium and other miscellaneous chemicals were purchased from Sigma (St Louis, MO). Polymorphoprep™ was purchased from Greiner Bio-One, Inc. (Longwood, FL). Dulbecco's modification of Eagle's Medium (DMEM), Ham's F-12 medium and penicillin–streptomycin (P-S) were purchased from Cellgro (Herndon, VA). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT). The CellTiter 96® AQueous One Solution Cell Proliferation Assay was purchased from Promega (Madison, WI). High strength analytical grade agarose was purchased from Bio-Rad (Hercules, CA). Ionagar No. 2 was purchased from Oxoid (London, UK). 5-(and-6)-Carboxy-2',7'-dichlorodihydrofluorescein diacetate (carboxy-H₂DCFDA) was purchased from Molecular Probes (Carlsbad, CA).

Water soluble extraction of Pycnogenol®. Pyc was incubated at 56 °C for 5 h in double distilled water, allowed to cool to room temperature and filtered using a Steriflip® Vacuum Filtration System (0.22 µm Durapore PVDF membrane; Millipore Corporation, Bedford, MA).

Cell culture and treatments. Two cell cultures of human origin were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. OSE2a (immortalized normal ovarian epithelial) and GC1a (immortalized normal ovarian granulosa) cell cultures were donated by Dr Hitoshi Okamura at Kumamoto University, Japan (Okamura *et al.*, 2003). The cell lines were maintained in a 1:1 mixture of DMEM and Ham's F-12 medium supplemented with 10% FBS and 100 IU/mL P-S. In preparation for either talc or Pyc + talc treatments, each cell line was seeded (1×10^5 cells/ml) and grown to 80% confluence, unless otherwise specified. Cells were incubated with 0–500 µg/mL talc from 24 to 120 h; or 0–500 µg/mL Pyc for 24 h followed by 5 µg/mL talc for 24 or 72 h.

Neutrophil isolation and culture. Peripheral blood polymorphonuclear neutrophils (PMN) and monocytes were obtained from heparinized venous blood from healthy volunteers (protocol approved by Loma Linda University Institutional Review Board for Human Studies) and isolated by Polymorphoprep™ density gradient centrifugation followed by the hypotonic lysis of erythrocytes. The purity of PMNs was determined by Giemsa staining as greater than 95%. Purified cells were suspended at 5×10^5 cells/mL in RPMI-1640 containing 2 mM L-glutamine, 1 mM sodium pyruvate, supplemented with 10% FBS and 100 IU/mL P-S; and treated with varying concentrations of talc for 24 or 72 h. ROS generation was detected as detailed below.

Cell viability assay. The CellTiter 96® AQueous One Solution Cell Proliferation Assay was used to measure cell viability (Buz'Zard and Lau, 2004). The MTS [3-(4,5-dimethylthiazolyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt] solution was used according to manufacturer's instructions. The absorbance was read at 490 nm using a model 3550 Microplate Reader (Bio-Rad). The percent cell viability was calculated as the absorbance of the treated cells divided by the absorbance of the untreated-control cells multiplied by 100.

Neoplastic transformation assay. A characteristic of cancer cells is their ability to grow and to divide when held in suspension without attachment or with minimal attachment to a rigid surface (Leung *et al.*, 2004). Thus, growth in soft agar demonstrates *in vitro* transformation of cells to their neoplastic counterparts (Morales *et al.*, 2003). After 72 h of incubation in the presence of talc; or in the presence of 0–500 µg/mL Pyc for 24 h followed by 5 µg/mL talc for 72 h, cells were collected, washed and suspended in 0.35% agarose at 5000 cells/well and layered on top of a base of 0.5% agar. The plates were incubated at 37 °C in a humidified incubator for 14 days. The cells were stained with 0.005% crystal violet and colonies were counted using an inverted microscope (Cory *et al.*, 1987).

Reactive oxygen species (ROS) detection. Carboxy-H₂DCFDA is a non-fluorescent dye that permeates the cells where it is deacetylated by viable cells to 2',7'-dichlorofluorescein (DCFH), which is then oxidized to fluorescent 2',7'-dichlorofluorescein (DCF) by endogenous hydrogen peroxide (H₂O₂) (Wan *et al.*, 1993). The cells were seeded in Optilux™ 96-well plates (BD Falcon, Bedford, MA) and treated with 0 to 500 µg/mL Pyc for 24 h. H₂O₂ (100 µM) was used as a positive control. Carboxy-H₂DCFDA (5 µM) was added and incubated for 1 h. The fluorescence intensity (excitation 485 nm/emission 530 nm) was measured as arbitrary fluorescent units (AFU) using a model 7600 Microplate Fluorometer (Cambridge Technology, Inc., Watertown, MA). The percent AFU (a.k.a. % ROS generation) was calculated as the 'treated cell-AFU' divided by the 'untreated cell-AFU' multiplied by 100. Immediately following the fluorescence detection, the fluorescence intensity was normalized by the cell viability assay.

Statistical analysis. Data were reported as mean ± SE. Statistical analysis was performed with the Student's paired *t*-test.

RESULTS

All experiments were performed a minimum of three times with reproducible results.

Effect of talc on cell viability of normal ovarian cells

Talc caused a bell-shaped curve response in OSE2a cells, with a statistically significant increase seen at 5 µg/mL (24 h) and a statistically significant decrease at 200 µg/mL (72 h) and 500 µg/mL (24 and 72 h) (Fig. 1).

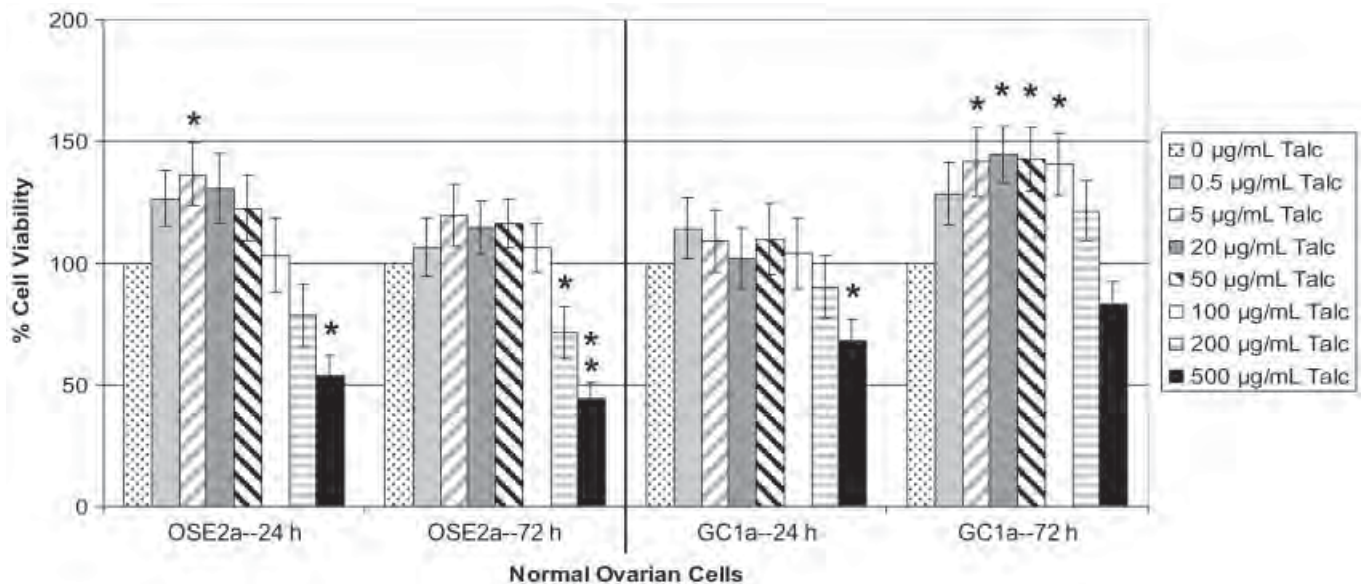


Figure 1. Effect of talc on the cell viability of ovarian cells. Normal ovarian epithelial (OSE2a) and normal ovarian stromal (GC1a) cells were treated with various concentrations of talc for 24 and 72 h. Cell viability was measured by the MTS assay and the percent cell viability was calculated as the absorbance of the treated cell divided by the absorbance of the untreated-control cells multiplied by 100. Each data point represents mean \pm SE of five determinations. Statistical significance was determined by the Student's paired *t*-test. * $p < 0.05$, ** $p < 0.01$ comparing the treatment with the respective untreated control.

Also seen in Fig. 1, talc caused a bell-shaped curve response in GC1a cells, with a statistically significant increase seen at 5, 20, 50 and 100 $\mu\text{g/mL}$ (72 h) and a statistically significant decrease at 500 $\mu\text{g/mL}$ (24 h).

Effect of talc on neoplastic transformation of normal ovarian cells

Since the ability to grow suspended in soft agar is a characteristic of cells being transformed to their

neoplastic counterparts (Leung *et al.*, 2004; Morales *et al.*, 2003), the study determined whether talc would be able to induce such a transformation. As shown in Fig. 2, talc caused a statistically significant increase in the number of transformed colonies in the OSE2a cells at 5 and 20 $\mu\text{g/mL}$ talc and in the GC1a cells at 5, 20 and 100 $\mu\text{g/mL}$ talc, compared with the untreated control. An exception was seen in the 100 $\mu\text{g/mL}$ talc treatment in the OSE2a cells in which the number of transformed colonies was reduced significantly.

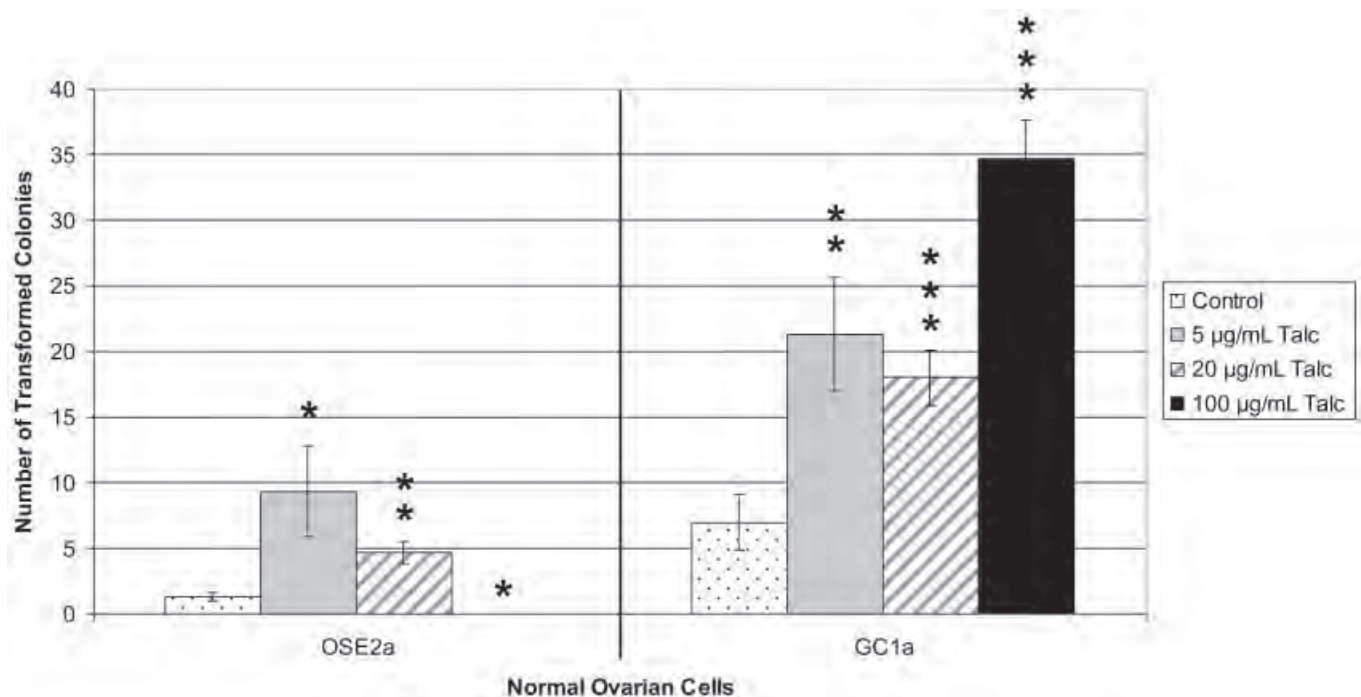


Figure 2. Neoplastic transformation of ovarian cells by talc. Normal ovarian epithelial (OSE2a) and normal ovarian stromal (GC1a) cells were incubated with various concentrations of talc for 72 h, collected, washed, seeded in soft agar suspension and grown for 14 days before colonies were counted. Each data point represents mean \pm SE of three determinations. Statistical significance was determined by the Student's paired *t*-test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ comparing the treatment with the respective untreated control (0 $\mu\text{g/mL}$ talc).

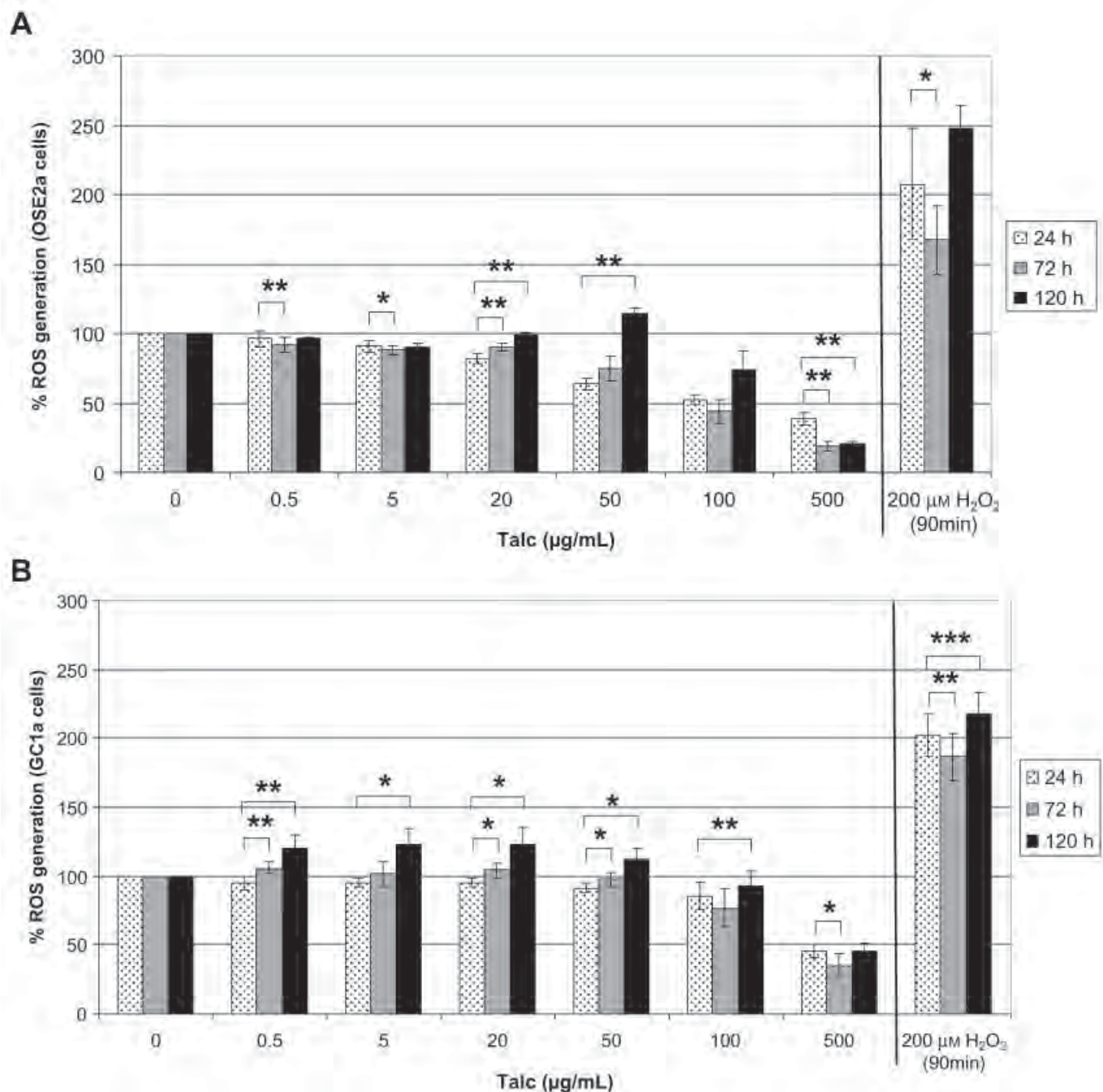


Figure 3. ROS generation of ovarian cells in response to talc treatments. Normal ovarian epithelial (OSE2a) and normal ovarian stromal (GC1a) cells were treated with various concentrations of talc for 24, 72 and 120 h and H₂O₂ during the last 90 min of each respective time point. H₂O₂ was used as a positive control for this assay. Fluorescence intensity were measured as arbitrary fluorescent units (AFU) at ex 485 nm/em 530 nm and normalized with the cell viability assay. Percent AFU (a.k.a. % ROS generation) was calculated as the average AFU of the treated cell divided by the average AFU of the untreated-control cells multiplied by 100. (A) ROS generation in OSE2a cells in response to talc treatments. (B) ROS generation in GC1a cells in response to talc treatments. Each data point represents mean \pm SE of three determinations. Statistical significance was determined by the Student's paired *t*-test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ comparing the treatment with the respective untreated control (as demonstrated by the horizontal brackets).

Effect of talc on ROS generation in normal ovarian cells

Talc caused an initial dose-dependent decrease in ROS generation (24 h) which increased with time in OSE2a cells (Fig. 3A). However, as time increased, ROS generation rebounded and increased compared with the values at 24 h. A statistically significant increase was seen at 20 µg/mL (72 and 120 h) and 50 µg/mL (120 h). Talc also caused an initial dose-dependent decrease in ROS generation (24 h) in GC1a cells (Fig. 3B), but

ROS generation increased with time in the talc treated cells. A statistically significant increase was seen with 0.5, 20 and 50 µg/mL (72 and 120 h), as well as 5 and 100 µg/mL (120 h) compared with the respective 24 h value.

Effect of talc on ROS generation in PMN

Since oxidative stress is often a component of the tumor microenvironment (Valko *et al.*, 2004), the study tested whether talc was capable of inducing ROS generation

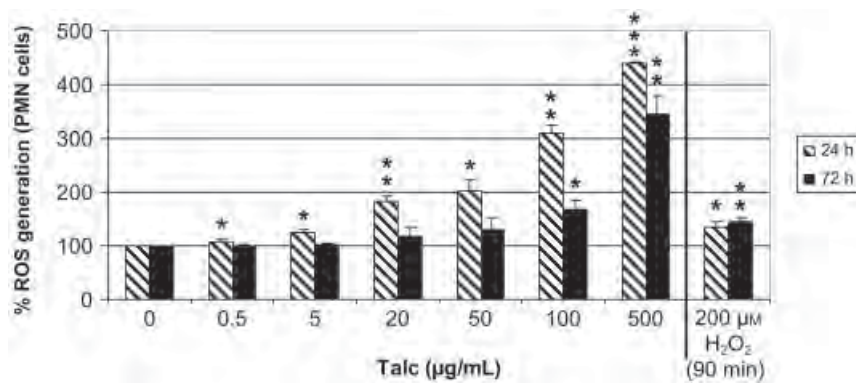


Figure 4. ROS generation of polymorphonuclear neutrophils (PMN) in response to talc treatments. PMNs were treated with various concentrations of talc for 24 and 72 h and H₂O₂ during the last 90 min of each respective time point. H₂O₂ was used as a positive control for this assay. Fluorescence intensity were measured as arbitrary fluorescent units (AFU) at ex 485 nm/em 530 nm and normalized with the cell viability assay. Percent AFU (a.k.a. % ROS generation) was calculated as the average AFU of the treated cell divided by the average AFU of the untreated-control cells multiplied by 100. ROS generation of PMNs in response to talc treatments. Each data point represents mean \pm SE of three determinations. Statistical significance was determined by the Student's paired *t*-test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ comparing the treatment with the respective untreated control.

in human PMNs. Talc caused a dose-dependent increase in ROS generation at both time points (Fig. 4). The increase was statistically significant at 0.5, 5, 20, 50 µg/mL (24 h) and 100 and 500 µg/mL (24 and 72 h). The maximum ROS generation was seen at 500 µg/mL and was increased over 4-fold at 24 h and 3.5-fold at 72 h, compared with the respective untreated cells.

(Fig. 5A). Pretreatment with Pyc caused a general decrease in cell viability in the GC1a cells (Fig. 5B) compared with the respective untreated GC1a cells. One exception is that of a slight, but statistically significant, increase in cell viability at 100 µg/mL Pyc + 5 µg/mL talc (72 h) compared with the respective untreated GC1a cells (Fig. 5B).

Effect of pretreatment with Pyc on talc-induced cell viability changes in normal ovarian cells

Pretreatment with Pyc did not cause a statistically different change in cell viability in the OSE2a cells

Effect of pretreatment with Pyc on talc-induced neoplastic transformation of normal ovarian cells

Pretreatment with Pyc decreased the number of neoplastically transformed colonies induced by talc in

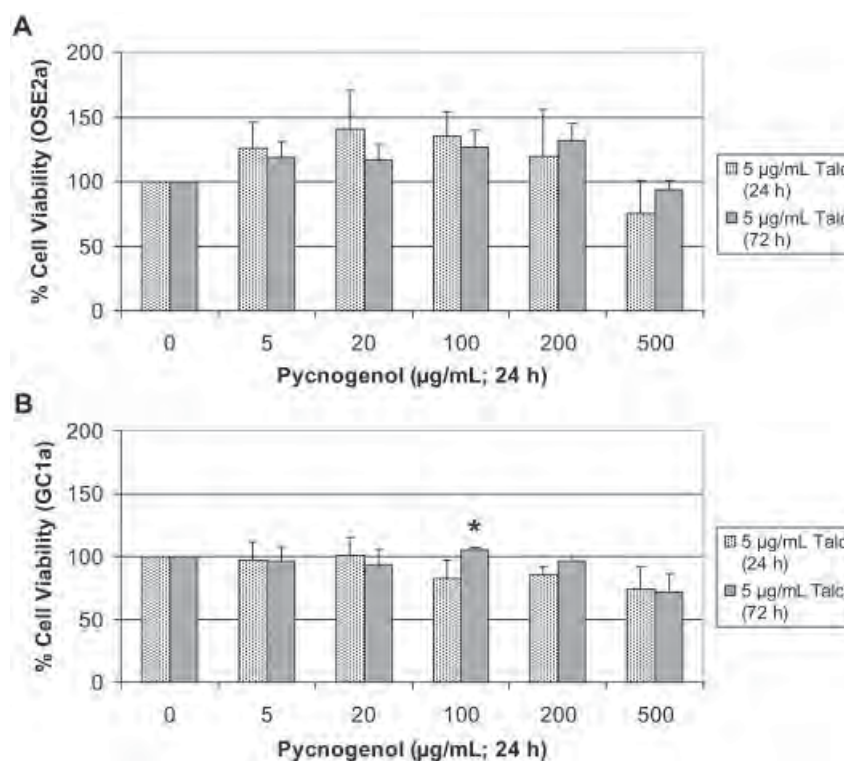


Figure 5. Effect of Pyc + talc treatments on the cell viability of ovarian cells. Normal ovarian epithelial (OSE2a) and stromal (GC1a) cells were treated with 0–500 µg/mL Pyc for 24 h followed by 5 µg/mL talc for 24 and 72 h. Cell viability was measured by the MTS assay and percent cell viability was calculated as the absorbance of the treated cell divided by the absorbance of the untreated-control cells multiplied by 100. (A) OSE2a cells. (B) GC1a cells. Each data represent mean \pm SE of four determinations. Statistical significance was determined by the Student's paired *t*-test. * $p < 0.05$ comparing the treatment with the respective untreated control.

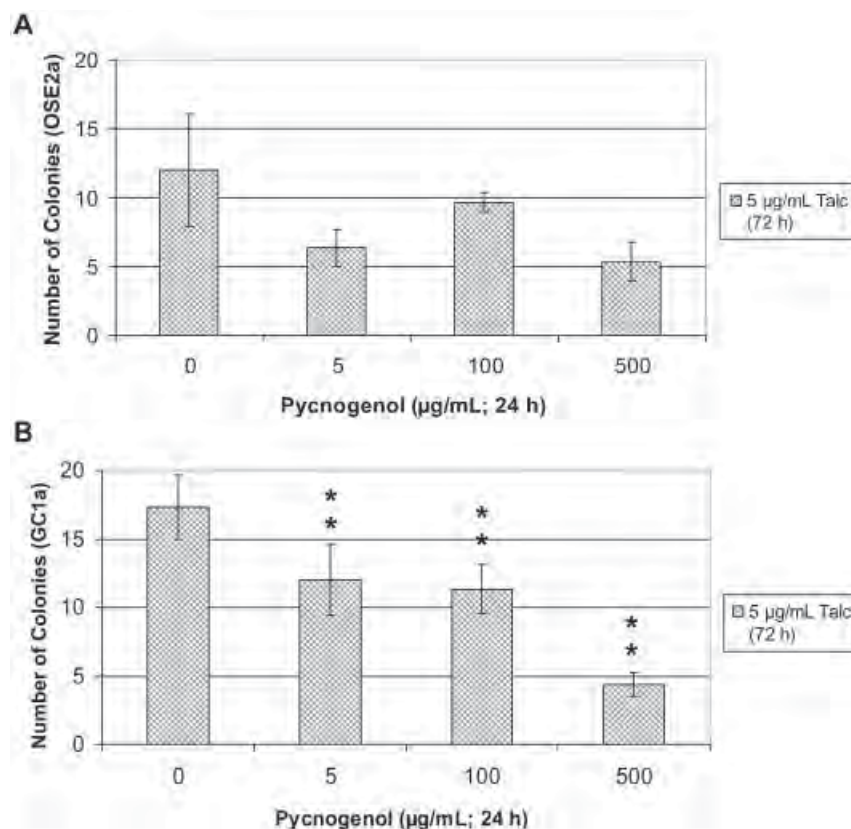


Figure 6. Pyc-induced protection against neoplastic transformation of ovarian cells by talc. Normal ovarian epithelial (OSE2a) and stromal (GC1a) cells were incubated with 0–500 $\mu\text{g/mL}$ Pyc for 24 h followed by 5 $\mu\text{g/mL}$ talc for 72 h, collected, washed, seeded in soft agar suspension and grown for 14 days before colonies were counted. (A) OSE2a cells. (B) GC1a cells. Each data represent mean \pm SE of three determinations. Statistical significance was determined by the Student's paired *t*-test. ** $p < 0.01$ comparing the treatment with the respective control.

the OSE2a cells, but not in a statistically significant manner (Fig. 6A). Pretreatment with Pyc (5, 100 and 500 $\mu\text{g/mL}$; 24 h) caused a statistically significant decrease in the number of talc-induced neoplastically transformed colonies in the GC1a cells (Fig. 6B).

Effect of pretreatment with Pyc on talc-induced ROS generation in normal ovarian cells

Pretreatment with Pyc caused a statistically significant decrease in ROS generation at 5, 20, 50, 100 and 200 $\mu\text{g/mL}$ Pyc + 5 $\mu\text{g/mL}$ talc (24 h); and 500 $\mu\text{g/mL}$ Pyc + 5 $\mu\text{g/mL}$ talc (24 and 72 h) in the OSE2a cells (Fig. 7A). Pretreatment with Pyc caused a statistically significant decrease in ROS generation at 5, 20, 50, 200 and 500 $\mu\text{g/mL}$ Pyc + 5 $\mu\text{g/mL}$ talc (24 h) in the GC1a cells (Fig. 7B). Pretreatment with Pyc caused a statistically significant decrease in ROS generation at 5, 20, 50, 100, 200 and 500 $\mu\text{g/mL}$ Pyc + 5 $\mu\text{g/mL}$ talc (72 h) in the GC1a cells (Fig. 7B). The decrease seen at 100 $\mu\text{g/mL}$ Pyc + 5 $\mu\text{g/mL}$ talc (24 h) was not statistically significant (Fig. 7B).

DISCUSSION

Cancer development is a multi-step process comprising a series of cellular and molecular changes that are mediated by various endogenous and exogenous stimuli, such as aberrantly expressed ROS (Storz, 2005).

Although ROS are a byproduct of endogenous biochemical processes, ROS (such as H_2O_2) at high concentrations or expressed in a chronic nature can damage cellular macromolecules and contribute to neoplastic transformation and tumor growth (Nicco *et al.*, 2005). A characteristic of neoplastically transformed cells is their ability to grow and to divide when held in suspension without attachment or with minimal attachment to a rigid surface (Leung *et al.*, 2004; Morales *et al.*, 2003). Our data show that talc not only increased cell viability (Fig. 1A), but also caused an increase in transformed cells in both the stromal and epithelial ovarian cells by their ability to grow, divide and form colonies while being suspended in soft agar (Fig. 2A).

It is known that substances that raise the intracellular level of H_2O_2 are able to trigger normal cell proliferation and abolish tumor cell proliferation (Ness and Cottreau, 1999; Nicco *et al.*, 2005). In normal cells, the basal level of H_2O_2 is low and its increase is initially associated with cell growth. H_2O_2 at high concentrations or expressed in a chronic nature in normal cells, can damage cellular macromolecules and contribute to neoplastic transformation and tumor growth (Nicco *et al.*, 2005). In this study, talc was shown to increase the ROS generation, after an initial suppression, in a time-dependent manner in the normal stromal cells (Fig. 3B) and less strongly in the normal epithelial cells (Fig. 3A).

Recent studies have expanded the concept that inflammation is a critical component of tumor progression. The neoplastic process (proliferation, survival and

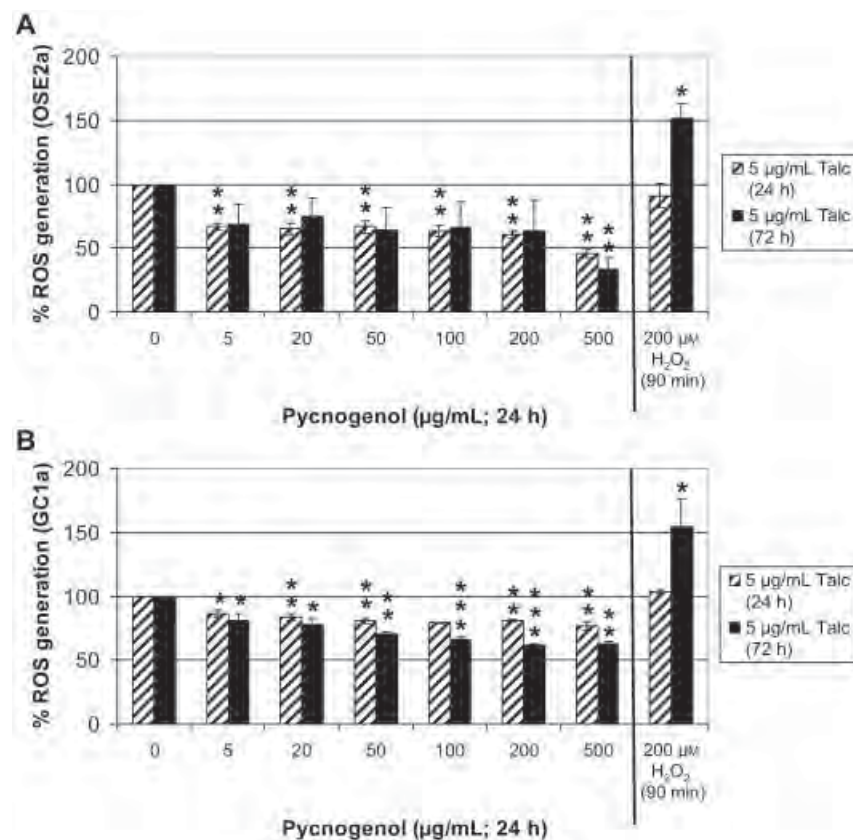


Figure 7. ROS generation of ovarian cells in response to Pyc + talc. Normal ovarian epithelial (OSE2a) and stromal (GC1a) cells were treated with 0–500 µg/mL Pyc for 24 h, followed by 5 µg/mL talc for 24 or 72 h and H₂O₂ (the last 90 min of each time point) as a positive control. Fluorescence intensity (AFU) was measured at ex 485 nm/em 530 nm and normalized by cell viability assay. The percent ROS generation was calculated as the average AFU of treated divided by AFU of untreated-control multiplied by 100. (A) OSE2a cells. (B) GC1a cells. Each data point represents mean ± SE of three determinations. Statistical significance was determined by the Student's paired *t*-test. * *p* < 0.05, ** *p* < 0.01 and *** *p* < 0.001 comparing the treatment with the respective untreated control.

migration) is linked with the tumor microenvironment and synchronized with inflammatory cells (Valko *et al.*, 2004). Polymorphonuclear neutrophils and macrophages are a main source of exogenous ROS in that they release large quantities of ROS in response to a variety of stimuli. This exogenously produced ROS is crucial in the innate immune system of the host for killing invading bacteria but may also be responsible for tissue injury, when expressed excessively or inappropriately (Lewis and Pollard, 2006). Inflammatory cells are prominent in the stromal compartment of virtually all types of malignancy. These highly versatile cells respond to the presence of stimuli in different parts of tumors (Balkwill and Mantovani, 2001). In an *in vitro* study of rat cells, both macrophages and neutrophils were found to be mutagenic in response to alpha-quartz dust, talc and diesel soot; however, neutrophils appeared to have a greater mutagenic effect (Driscoll *et al.*, 1997). This study found that talc not only increased the ROS generation in the ovarian cells (Fig. 3), but also increased the expression of ROS by the neutrophils (Fig. 4).

Talc has been shown to be ubiquitous in our modern environment (Bremmell and Addai-Mensah, 2005) despite concerns raised about its safety (Janssen, 2004), its role as a possible carcinogen (Cramer *et al.*, 1999; Wong *et al.*, 1999), and its known ability to cause irritation and inflammation (Holthouse and Chleboun, 2001). The data show that talc is capable of increasing

cell proliferation, inducing neoplastic transformation of both the normal stromal and epithelial ovarian cells *in vitro*; and increasing ROS generation in these cells as well as the PMN cells.

Cancer chemoprevention is regarded as an efficient strategy to prevent cancer. The most useful cancer chemopreventive compounds will have minimal long-term toxicity, while significantly reducing tumor incidence, delaying tumor onset or preventing tumor progression (Kapadia *et al.*, 2003). It was hypothesized that Pyc, shown to induce apoptosis in various malignant cells (Huang *et al.*, 2005; Huynh and Teel, 2000), could prevent talc-induced neoplastic transformation of normal ovarian cells. It was recently shown that Pyc selectively induced cell death in established malignant ovarian germ cells *in vitro* (Buz'Zard and Lau, 2004). The present study showed that Pyc was capable of inhibiting the above mentioned talc-induced changes. Pretreatment with Pyc prevented the characteristic talc-induced increase in cell viability of GC1a cells (Fig. 5B). Pretreatment with Pyc was able to decrease the ROS generation compared with the respective controls both in a dose- and time-dependent manner (Fig. 7). The data show that pretreatment with Pyc reduced the number of talc-induced transformed colonies in both cell lines (Fig. 6). In the GC1a cells, the decrease in the number of transformed colonies was statistically significant at all concentrations of Pyc (Fig. 6B).

In conclusion, our *in vitro* data suggest that: (1) talc may contribute to ovarian carcinogenesis in humans by way of inducing aberrant ROS generation and (2) Pyc reduces talc-induced neoplastic transformation of ovarian cells. Taken together, Pyc may prove to be a chemopreventative agent against ovarian carcinogenesis.

Acknowledgements

This study was partially supported by a grant from Horphag Research, Geneva, Switzerland (Otherwise, there is no conflict of interest). We thank Dr Hitoshi Okamura for the cell lines. We thank El Chay for his guidance. We thank Vandana Shah, Marsha Yarnell and Christina Wright for their assistance.

REFERENCES

- American Cancer Society. 2000. *Ovarian Cancer*, 1–29.
- Balkwill F, Mantovani A. 2001. Inflammation and cancer: back to Virchow? *Lancet* **357**: 539–545.
- Bremmell KE, Addai-Mensah J. 2005. Interfacial-chemistry mediated behavior of colloidal talc dispersions. *J Colloid Interface Sci* **283**: 385–391.
- Buz'Zard AR, Lau BHS. 2004. Selective toxicity of Pycnogenol for malignant ovarian germ cells *in vitro*. *Int J Cancer Prev* **1**: 207–212.
- Cory S, Bernard O, Bowtell D, Schrader S, Schrader JW. 1987. Murine c-myc retroviruses alter the growth requirements of myeloid cell lines. *Oncogene Res* **1**: 61–76.
- Cramer DW, Liberman RF, Titus-Ernstoff L *et al.* 1999. Genital talc exposure and risk of ovarian cancer. *Int J Cancer* **81**: 351–356.
- Driscoll KE, Deyo LC, Carter JM, Howard BW, Hassenbein DG, Bertram TA. 1997. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. *Carcinogenesis* **18**: 423–430.
- Hamilton TC, Fox H, Buckley CH, Henderson WJ, Griffiths K. 1984. Effects of talc on the rat ovary. *Br J Exp Pathol* **65**: 101–106.
- Hanna L, Adams M. 2006. Prevention of ovarian cancer. *Best Pract Res Clin Obstet Gynaecol* **20**: 339–362.
- Henderson WJ, Hamilton TC, Baylis MS, Pierrepont CG, Griffiths K. 1986. The demonstration of the migration of talc from the vagina and posterior uterus to the ovary in the rat. *Environ Res* **40**: 247–250.
- Holthouse DJ, Chleboun JO. 2001. Talc serodesis – report of four cases. *J R Coll Surg Edinb* **46**: 244–245.
- Huang WW, Yang JS, Lin CF, Ho WJ, Lee MR. 2005. Pycnogenol induces differentiation and apoptosis in human promyeloid leukemia HL-60 cells. *Leukemia Res* **29**: 685–692.
- Huynh HT, Teel RW. 2000. Selective induction of apoptosis in human mammary cancer cells (MCF-7) by pycnogenol. *Anticancer Res* **20**: 2417–2420.
- Janssen JP. 2004. Is thoracoscopic talc pleurodesis really safe? *Monaldi Arch Chest Dis* **61**: 35–38.
- Kapadia GJ, Azuine MA, Sridhar R *et al.* 2003. Chemoprevention of DMBA-induced UV-B promoted, NOR-1-induced TPA promoted skin carcinogenesis, and DEN-induced phenobarbital promoted liver tumors in mice by extract of beetroot. *Pharmacol Res* **47**: 141–148.
- Knasmuller S, Verhagen H. 2002. Impact of dietary factors on cancer causes and DNA integrity: new trends and aspects. *Food Chem Toxicol* **40**: 1047–1050.
- Leung DW, Tompkins C, Brewer J *et al.* 2004. Phospholipase C delta-4 overexpression upregulates ErbB1/2 expression, Erk signaling pathway, and proliferation in MCF-7 cells. *Mol Cancer* **3**: 15.
- Lewis CE, Pollard JW. 2006. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* **66**: 605–612.
- Morales CP, Gandia KG, Ramirez RD, Wright WE, Shay JW, Spechler SJ. 2003. Characterisation of telomerase immortalised normal human oesophageal squamous cells. *Gut* **52**: 327–333.
- Nelson AB, Lau BHS, Ide N, Rong Y. 1998. Pycnogenol inhibits macrophage oxidative burst, lipoprotein oxidation and hydroxyl radical-induced DNA damage. *Drug Dev Indust Pharm* **24**: 139–144.
- Ness RB, Cottreau C. 1999. Possible role of ovarian epithelial inflammation in ovarian cancer. *J Natl Cancer Inst* **91**: 1459–1467.
- Nicco C, Laurent A, Chereau C, Weill B, Batteux F. 2005. Differential modulation of normal and tumor cell proliferation by reactive oxygen species. *Biomed Pharmacother* **59**: 169–174.
- Okamura H, Katabuchi H, Ohba T. 2003. What we have learned from isolated cells from human ovary? *Mol Cell Endocrinol* **202**: 37–45.
- Storz P. 2005. Reactive oxygen species in tumor progression. *Front Biosci* **10**: 1881–1896.
- Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem* **266**: 37–56.
- Van Dyke K, Patel S, Vallyathan V. 2003. Lucigenin chemiluminescence assay as an adjunctive tool for assessment of various stages of inflammation: a study of quiescent inflammatory cells. *J Biosci* **28**: 115–119.
- Wan CP, Myung E, Lau BH. 1993. An automated microfluorometric assay for monitoring oxidative burst activity of phagocytes. *J Immunol Methods* **159**: 131–138.
- Wei ZH, Peng QL, Lau BHS. 1997. Pycnogenol enhances endothelial cell antioxidant defenses. *Redox Rep* **3**: 219–224.
- Werebe EC, Pazetti R, Milanez DC, Jr *et al.* 1999. Systemic distribution of talc after intrapleural administration in rats. *Chest* **115**: 190–193.
- Wong C, Hempling RE, Piver MS, Natarajan N, Mettlin CJ. 1999. Perineal talc exposure and subsequent epithelial ovarian cancer: a case-control study. *Obstet Gynecol* **93**: 372–376.

Exhibit 95



Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

The primary role of iron-mediated lipid peroxidation in the differential cytotoxicity caused by two varieties of talc nanoparticles on A₅₄₉ cells and lipid peroxidation inhibitory effect exerted by ascorbic acid

Mohd Javed Akhtar^a, Sudhir Kumar^b, Ramesh Chandra Murthy^c, Mohd Ashquin^a, Mohd Imran Khan^a, Govil Patil^a, Iqbal Ahmad^{a,*}

^a Fibre Toxicology Division, Indian Institute of Toxicology Research (CSIR), Lucknow 226 001, UP, India

^b Department of Zoology, University of Lucknow, Lucknow 226 001, UP, India

^c Analytical Chemistry Division, Indian Institute of Toxicology Research (CSIR), Lucknow 226 001, UP, India

ARTICLE INFO

Article history:

Received 8 October 2009

Accepted 3 March 2010

Available online 10 March 2010

Keywords:

Talc nanoparticles

Cytotoxicity

Lipid peroxidation

Reactive oxygen species

Glutathione

Oxidative stress

Iron contamination

ABSTRACT

Talc particles, the basic ingredient in different kinds of talc based cosmetic and pharmaceutical products, pose a health risk to pulmonary and ovarian systems due to domestic and occupational exposures. Two types of talc nanoparticles depending on the source of geographical origin indigenous and commercial talc nanoparticles were assessed for their potential *in vitro* toxicity on A₅₄₉ cells; along with indigenous conventionally used microtalc particles. Cell viability, determined through live/dead staining and 3 (4,5 dimethyl thiazol 2 yl) 2,5 diphenyl tetrazolium bromide (MTT) assay, decreased as a function of concentration, origin and size of particles. Both varieties of talc nanoparticles differentially induced lipid peroxidation (LPO), which was correlated with the pattern of lactate dehydrogenase (LDH) leakage, reactive oxygen species (ROS) generation, and glutathione (GSH) depletion. Relatively higher cytotoxicity of indigenous nanotalc could be attributed to its higher content of iron as compared to commercial nanotalc. The known scavenger of ROS, i.e. ascorbic acid significantly inhibited LPO induction due to talc particles. Data suggest that nanotalc toxicity on A₅₄₉ cells was mediated through oxidative stress, wherein role of iron mediated LPO was much pronounced in differential cytotoxicity.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Talc is a magnesium silicate mineral with chemical formula written as 3MgO·4SiO₂·H₂O which corresponds to 4.8% H₂O, 31.7% MgO, and 63.5% SiO₂. It is chemically inert to acids and alkalis and can withstand temperatures up to 1300 °C. In pulverized form it is whiter in appearance. Talc is valued for its extreme softness, smoothness, high lubricating and hiding power and ability to absorb oil and grease. Talc is, therefore, used by organized sector of industries because of its valuable properties. Pulverized talc has wide industrial applications in cosmetics as body and face powder; filler in rubber, textile, plastic, asbestos products, polishes and soaps; as a loading agent for paper of all kinds; used in pharmaceuticals as a carrier of insecticidal and pesticidal dusts.

Since, talc products are marketed in a multitude of grades which have physical or functional characteristics especially suited for particular applications and products, so occupational and con-

sumer exposures to talc are complex. Talc miners have shown higher rates of lung cancer and other respiratory illnesses from exposure to industrial grade talc, which contains dangerous silica and asbestos (Hollinger, 1990; National Toxicology Program, 1993). The common household hazard posed by talc is inhalation of baby powder by infants (Hollinger, 1990). Talc particles have been found to be translocated after intrapleural administration in rats (Werebe and Pazetti, 1999). Talc particles are able to move through the human reproductive system and become imbedded in the lining of the ovary. Researchers have found talc particles in ovarian tumors and have found that women with ovarian cancer have used talcum powder in their genital area more frequently than healthy women (Henderson et al., 1971; Harlow et al., 1992; Harlow and Hartge, 1995). Numerous studies have shown a strong link between frequent use of talc in the female genital area and ovarian cancer (Heller et al., 1996; Chang and Risch, 1997; Cook et al., 1997; Cramer et al., 1999; Mills et al., 2004; Wild, 2006). In an epidemiologic study aimed to analyze the interactions between talc use and genes involved in detoxification pathway, (viz: glutathione S transferase M1 (GSTM1), glutathione S transferase T1 (GSTT1), and N acetyltransferase 2 (NAT2), suggest that women with certain genetic variants may have a higher risk of

* Corresponding author. Address: Fibre Toxicology Division, Indian Institute of Toxicology Research, P.O. Box No. 80, Mahatma Gandhi Marg, Lucknow 226 001, UP, India. Tel.: +91 522 2620207/2227586; fax: +91 522 2628227.

E-mail addresses: iqbal@iitr.res.in, ahmadi@sify.com (I. Ahmad).

ovarian cancer associated with genital use of talc (Gates et al., 2008).

Nanopowder of talc is a recent introduction and is used for improving quality of many industrial products. Nanopowder of talc is being used in plastics for higher strength and stiffness, better thermal and creep resistance; in papers for higher opacity, better gloss and printing quality; in cosmetics and paints for better gloss, smoother surface, resistance to water and cracking, etc. Owing to their unique nano size, nanoparticles are provided with many special physicochemical properties, and thereby may yield extraordinary hazards for human health (Donaldson et al., 2002; Kipen and Laskin, 2005; Holsapple et al., 2005; Nel et al., 2006; Borm et al., 2006). Since, talc with a multitude of physical and functional characteristics is used for particular applications, so occupational and consumer exposures to talc are likely to vary accordingly. Risk of occupational and environmental exposure to nanoparticles of talc has obviously increased.

Since, physical and functional characteristics of talc and other minerals depend, in part, from one geographical region/source to other, therefore, the first objective of the present study was to evaluate cytotoxicity of talc nanoparticles from the two sources indigenous nanotalc (Indian origin) and commercial nanotalc (American origin) using human bronchoalveolar carcinoma derived cells (A₅₄₉). Indigenous micro scale talc particle was used for comparative size dependent toxicity with the two types of nanotalc. The second objective was to study the mechanism of cytotoxicity induced by talc nano and micro particles. In the present study, different types of talc particles were dispersed in the cell culture medium at varying concentrations and then exposed to cells. Cytotoxicity was measured by determining cell viability using MTT assay and live dead staining method. To elucidate the possible mechanisms of cytotoxicity, biomarkers for cytotoxicity and oxidative stress, namely lactate dehydrogenase (LDH) leakage in cell culture medium, reactive oxygen species (ROS) generation, intracellular reduced glutathione (GSH) level, and malondialdehyde (MDA) as an indicator of lipid peroxidation and membrane damage, were measured. Antioxidant, ascorbic acid, was used to delineate further the potential mechanism of oxidative stress and as a potential preventive measure. In the toxicity of minerals, the iron content has been a key factor, acting through Fenton reaction and the Haber Weiss cycle. Some metals like Fe, Pb, and Cr was measured in the talc from two sources. A role of differential amount of iron present in indigenous and commercial talc, in the perspective of cytotoxicity and oxidative stress has, therefore, also been established.

2. Materials and methods

2.1. Nanoparticles

Indigenous cosmetic grade talc was collected from Udaipur, Rajasthan, India and prepared into micro and nanoparticles. As a standard reference, Nanopowder of talc (i.e. commercial nanotalc) was purchased from (M.K. Impex Canada, Catalpa Road, Mississauga, Canada). As per the information provided by the supplier, the powder size was 70–120 nm and the country of origin was USA. For indigenous nanotalc a stone of talc was crushed into fine particles and fed into a ball mill (PM 100, Retsch, Germany) and grinded for 5 days at an alternative cycles of grinding (10 min) and halt (30 min) at 350 rpm using a mixture of different sizes of balls. The sizes of nanoparticles were measured by transmission electron microscopy (TEM) and found to be 80–130 nm. Indian talc particles (i.e. indigenous micro talc) 50–65 µm served as negative control for a comparative study on nanotoxicity of indigenous and commercial varieties of nanotalc.

2.2. Chemicals

Fetal bovine serum, Penicillin streptomycin, DMEM F 12 medium, HBSS was purchased from Invitrogen Co. (Carlsbad, CA, USA). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], NADH, Pyruvic acid, L-ascorbic acid, glutathione reduced (GSH), o-phthalaldehyde (OPT), 2,7'-dichlorofluorescein diacetate (DCFH DA), 1,1,3,3-tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA), sodium dodecyl sulphate (SDS), Na₂HPO₄, NaH₂PO₄, were obtained from Sigma Aldrich. Ultrapure DI water was prepared using a Milli Q system (Millipore, Bedford, MA, USA). All other chemicals used were of reagent grade.

2.3. Estimation of heavy metals in indigenous and commercial talc

Talc samples were digested in digesting mixture (HNO₃ and perchloric acid in a ratio of 4:1) for 24 h on hot plate in a fume hood. The digested samples were dissolved in 1% HNO₃ and filtered. The filtrate was used for metal analysis by atomic absorption spectroscopy (AAS). Before analysis, AAS was calibrated every time by running at least three standard concentration (1, 3 and 5 mg/L) of each metal. Values have been expressed as % metal content in talc samples.

2.4. Measurement of hydrodynamic size of nanotalc

These particles were suspended in complete cell culture media, ultrasonicated at 30 W for 2 min (Sonics Vibra Cell, India) and a dynamic light scattering (DLS—Malvern Instruments USA) performed for particle size distribution in culture media.

2.5. Cell culture and treatment with talc particles

The A₅₄₉ cell line has been established in permanent culture from a human lung adenocarcinoma (Lieber et al., 1976). *In vitro*, these cells are largely differentiated as alveolar epithelial cells, type II (Crouse et al., 1990). The A₅₄₉ cells were obtained from National Centre For Cell Science (NCCS), Pune, India. Cells were maintained in DMEM F 12 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin, and grown at 37 °C in a humidified, 5% CO₂ incubator. For the determination of GSH, MDA, and LDH levels, A₅₄₉ cells were plated into 75 cm² flasks at a density of 2.0×10^6 cells per flask in 12 ml culture medium and allowed to attach for 24 h. Then, the freshly dispersed talc nanoparticles suspensions in cell culture medium were prepared and diluted to appropriate concentrations (50, 100, and 200 µg/ml) and immediately applied to the cells in 15 ml culture medium. Cells not exposed to particles served as controls in each experiment. The selection of the 50–200 µg/ml dosage range of talc nanoparticles was based on a preliminary dose response study (data not shown). A dosage level lower than 50 µg/ml did not result cytotoxicity significantly. The 48 h exposure time was chosen for investigation; the responses at 24 h exposure were not as pronounced as that at 48 h. Therefore, all the data presented here is that of 48 h exposure. Throughout the studies presented in this paper, we utilized a particle dose of $20 \mu\text{g}/\text{cm}^2 = 100 \mu\text{g}/\text{ml}$.

2.6. Cell viability assay

Cytotoxicity was measured by determining cell viability using MTT assay and live dead staining method.

2.6.1. MTT assay

Cell proliferation/viability was assessed by the MTT assay as first described by Mossman (1983) and later modified by Hansen et al. (1989). This assay is based on the ability of viable cells, but

not of dead cells, to reduce soluble, yellow 3 (4,5 dimethyl thiazol 2 yl) 2,5 diphenyl tetrazolium bromide (MTT) into insoluble, blue formazan product. Briefly, around 10,000 A₅₄₉ cells per well were plated in 96 well microtiter plates in a 100 µl of medium. The next day, the medium was changed and the cells were treated with talc nanoparticles at 50 , 100 , and 200 µg/ml for 48 h. After the exposure time completed, the medium was aspirated off and 100 µl MTT laden media (0.5 mg MTT/ml of media without phenol red and serum, filtered through 0.22 µm filter) added and incubated for 2 h. The reaction was stopped and formazan crystal thus formed was solubilised by mixing an equal volume of stop mix solution containing 20% SDS in 50% N,N dimethylformamide and left overnight on a shaker. To minimize the interference in absorbance caused by previously dosed talc particles (at concentrations like 50 200 µg/ml obviously resulting in turbidity!), the plates were centrifuged at 3000 rpm for 5 min to settle down the particles and a clear 100 µl supernatant was transferred to other fresh wells of microtiter plate and then absorbance at 570 nm was taken by a microplate reader (Omega Fluostar). Following noncellular background (blank consisting of yellow MTT and stop mix solutions) subtraction, all data were normalized to the MTT conversion activity of media treated control cells. This value corresponds to 0% decrease in MTT conversion activity and represents 100% cell viability.

2.6.2. Live dead staining (trypan blue exclusion) assay

In addition to the MTT assay, the cell viability was also determined by the trypan blue exclusion method. The percentage of non stained live cells was evaluated using a haemocytometer. A total of 200 cells were counted for each measurement.

2.7. LDH leakage

The activity of cytoplasmic LDH released into the culture media was determined with the method described elsewhere (Wroblewski and LaDue, 1955; Welder et al., 1991). A 100 µl sample from the centrifuged culture media was collected after the cells were treated for 48 h. The LDH activity was assayed in 3.0 ml of reaction mixture with 100 µl of pyruvic acid (2.5 mg/ml phosphate buffer) and 100 µl of NADH (2.5 mg/ml phosphate buffer) and the rest of the volume adjusted with phosphate buffer (0.1 M, pH 7.4). The rate of NADH oxidation was determined by following the decrease in absorbance at 340 nm for 3 min at 30 s interval at 25 °C using a spectrophotometer (Thermo Spectronic). The amount of LDH released is represented as LDH activity (IU/L) in culture media.

2.8. Intracellular ROS measurement

The generation of intracellular ROS was measured using 2',7'-dichlorofluorescein diacetate (DCFH DA) probe (Wang and Joseph, 1999). DCFH DA passively enters the cell where it is broken down into cell impermeable, non fluorescent reduced dichlorofluorescein (DCFH) and diacetate by cellular esterases. Now DCFH becomes oxidized with intracellular ROS to form the highly fluorescent compound dichlorofluorescein (DCF) that may be cell permeable. Briefly, 10 mM DCFH DA stock solution made in dimethyl sulfoxide (DMSO) was diluted in culture medium without serum or other additive to yield a 100 µM working solution. After 48 h of exposure to talc nanoparticles, the cells in the 12 well plate were washed twice with HBSS and then incubated in 1 ml working solution of DCFH DA at 37 °C for 30 min. The cells were lysed in alkaline solution and centrifuged at 3000 rpm. A 200 µl supernatant was transferred to black 96 well plate and fluorescence was then read at 480 nm excitation and 520 nm emission using a microplate reader (Omega Fluostar). The intensity of untreated control well was assumed to be 100% and data is represented in percent of control.

2.9. Determination of intracellular GSH

The cellular content of GSH was quantified by the fluorometric assay of Hissin and Hilf (1976). After exposure, cells were lysed in 20 mM Tris (pH 7.0) by repeated cycles of freeze thaw and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was transferred to another tube and protein content was measured. For the determination of intracellular GSH, protein in this supernatant was precipitated at 1% perchloric acid and again centrifuged at 10,000 rpm for 5 min at 4 °C. Now 20 µl sample was mixed with 160 µl of 0.1 M phosphate 5 mM EDTA buffer, pH 8.3 and 20 µl o phthalaldehyde (OPT, 1 mg/ml in methanol) in a black 96 well plate. After 2 h incubation at room temperature in the dark, fluorescence was measured at an emission wavelength of 460 nm and an excitation wavelength of 355 nm, along with similarly prepared standards of GSH in 1% perchloric acid. Results are expressed as nmol GSH/mg of cellular protein.

2.10. Determination of thiobarbituric acid reactive substances (TBARS)

LPO was assessed by the TBARS assay, which detects mainly malondialdehyde (MDA), an end product of the peroxidation of polyunsaturated fatty acids and related esters. TBARS was measured by slight modification of the method of Ohkawa et al. (1979). Subconfluent cells were scraped in 75 cm² flasks, washed two times by isotonic trace element free Tris HCl buffer (400 mM, pH 7.3). A 200 µl aliquot of cell suspension was subsequently mixed with 800 µl of LPO assay cocktail containing (0.4% (w/v) thiobarbituric acid, 0.5% (w/v) SDS, 5% (v/v) acetic acid, pH 3.5 and incubated for 60 min at 95 °C. The sample was cooled using tap water and centrifuged at 5000 rpm for 5 min. The absorbance of the supernatants was read at 532 nm against a standard curve prepared using the MDA standard (10 mM 1,1,3,3 tetramethoxy propane in 20 mM Tris HCl, pH 7.4). Results were calculated as nmol of MDA/mg of cellular protein.

2.11. Addition of L ascorbic acid

To test the potential antioxidant effects afforded by ascorbic acid, 1.5 mM was applied to cell culture 30 min before exposure with particles. A dosage of 200 µg/ml of the two varieties of talc was then exposed for 48 h and MDA level was measured as illustrated above.

2.12. Estimation of protein

The total protein concentration was measured by the Bradford method (Bradford, 1976) using a ready to use Bradford reagent (Sigma Aldrich, USA) and bovine serum albumin as the standard.

2.13. Statistics

Data were expressed as the mean ± SD from three independent experiments. One way ANOVA and Dunnett's Multiple Comparison Test was applied using Graph Pad prism (Version 5.0) software for significance testing, using a *p* value ≤ 0.05.

3. Results

3.1. Iron contamination in talc samples

Indigenous and commercial talc samples were analyzed for contamination of heavy metals (Fe, Pb, and Cr). The results are given in Table 1. Indigenous talc contained almost 2.3 times higher iron level in comparison to commercial talc. Pb was not in detectable

Table 1
Metal contamination in talc samples.

Name of metal	% Metal content	
	Indigenous talc	Commercial talc
Fe	0.19	0.08
Cr	Not detectable	0.0046
Pd	Not detectable	Not detectable

limit in both the samples. However Cr was present in trace amount in commercial nanotalc.

3.2. Hydrodynamic size of talc nanoparticles in culture media

The size measured by a dynamic light scattering method was the particles hydrodynamic size, which indicates the extent of aggregation of particles in suspension. The measurements have been given in Table 2. Results show that aggregation occurred and the aggregation was not uniform.

3.3. The concentration, size, and origin dependent cytotoxicity of talc particles

The A_{549} cells were exposed with indigenous microtalc (50–65 μm) particles, indigenous talc nanoparticles (80–130 nm) and commercial talc nanoparticles (70–120 nm) for 48 h exposure, and the cell viability was assessed by MTT assay. Cell viability decreased as a function of concentration, size and geographical origin of particles. Cell viability decreased to 93.0%, 91.6%, and 83.6% for indigenous microtalc and 81.6%, 67.0%, and 47.30% for indigenous nanotalc and 88.3%, 77.6%, and 64.0% for commercial nanotalc particles when exposed at 50, 100, and 200 $\mu\text{g}/\text{ml}$, respectively (Fig. 1). Fig. 2 shows the results on cell viability obtained by trypan blue exclusion test for similar experiment. Cell viability decreased to about 93.0%, 90.6%, and 83.6% for indigenous microtalc and 83.6%, 73.6%, and 57.30% for indigenous nanotalc and 88.6%, 78.6%, and 69.6% for commercial nanotalc particles exposed at 50, 100, and 200 $\mu\text{g}/\text{ml}$, respectively. The IC_{50} s evaluated by MTT and trypan blue assay is given in Table 3.

3.4. Cell membrane damage

LDH release, a marker of cell membrane damage, was measured at 50, 100, and 200 $\mu\text{g}/\text{ml}$ for the 48 h exposure (Fig. 3). Following exposure to talc particles at concentrations mentioned above, the LDH activity in the culture media is increased in a concentration dependent manner and found to 18.1%, 32.9%, and 61.3%, respectively for indigenous microtalc and 99.2%, 193.6%, and 275.6%, respectively for indigenous nanotalc and 46.2%, 103.7%, and 178.7%, respectively for commercial nanotalc. The indigenous nanotalc induced a significantly higher ($p < 0.05$) cell membrane damage when compared with its micro scale size and commercial nanotalc for a particular concentration. For instance, 50, 100, and 200 $\mu\text{g}/\text{ml}$ exposure of indigenous nanotalc induced 1.4, 1.44, and 1.3 fold higher membrane damage when compared with the same concentrations of commercial nanotalc induced membrane damage. Similarly indigenous nanotalc induced membrane

Table 2
Actual and hydrodynamic sizes of Indigenous and Commercial nanotalc in culture media.

Type of nanoparticles	Actual size (nm)	Hydrodynamic size (nm)
Commercial nanotalc	70–120	800 \pm 100
Indigenous nanotalc	80–130	750 \pm 120

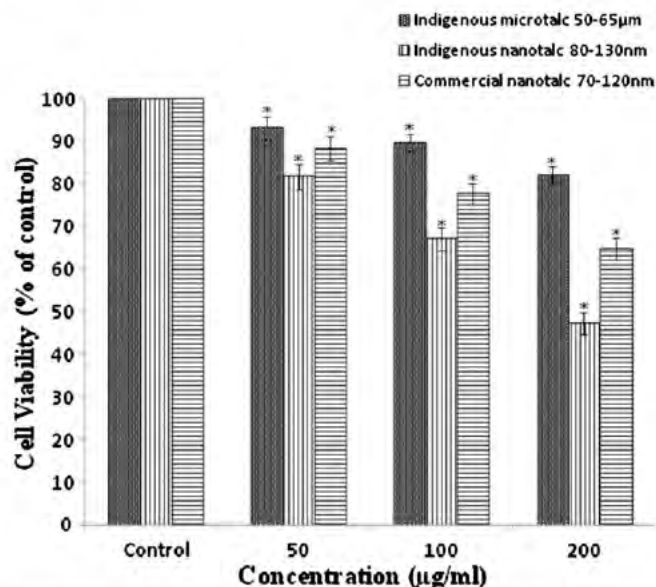


Fig. 1. Viability of A_{549} cells after 48-h exposure to indigenous microtalc, indigenous nanotalc and commercial nanotalc particles evaluated by MTT assay at indicated concentrations. Values are mean \pm SD from three independent experiments. Triplicates of each treatment group were used in each independent experiment. *Denotes a significant difference from the control ($p < 0.05$).

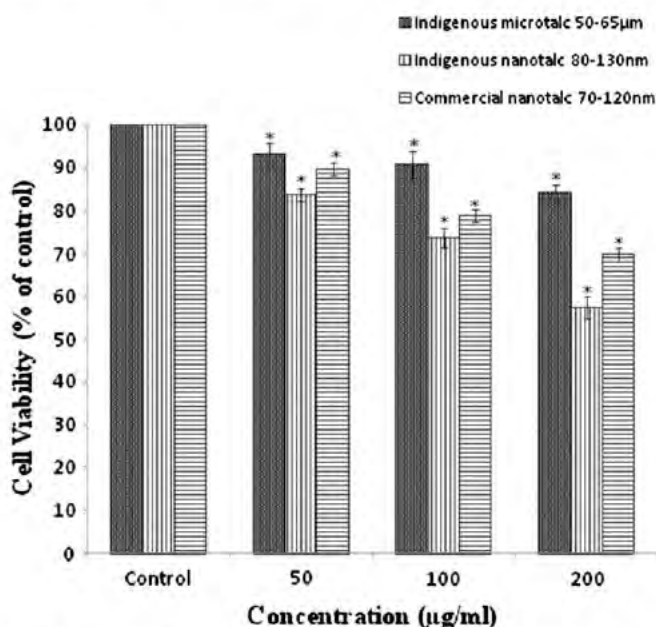


Fig. 2. Viability of A_{549} cells after 48-h exposure to indigenous microtalc, indigenous nanotalc and commercial nanotalc particles evaluated by trypan blue assay at indicated concentrations. Values are mean \pm SD from three independent experiments. Triplicates of each treatment group were used in each independent experiment. *Denotes a significant difference from the control ($p < 0.05$).

damage was 1.6, 2.2, and 2.3 times higher than that of indigenous microtalc.

3.5. ROS generation

The ability of talc micro and nanoparticles to induce intracellular oxidant production in A_{549} cells was assessed by measuring DCF fluorescence as a reporter of ROS generation. DCF fluorescence intensity significantly ($p < 0.05$) increased after 48 h exposure to all examined micro and nanoparticles at concentrations of 50,

Table 3
IC₅₀ values of different talc particles measured by MTT and trypan blue.

Types of talc nanoparticles	IC ₅₀ by MTT assay (μg/ml)	IC ₅₀ by trypan blue assay (μg/ml)
Indigenous microtalc (50–65 μm)	600	630
Indigenous nanotalc (80–130 nm)	190	255
Commercial nanotalc (70–120 nm)	277.5	325

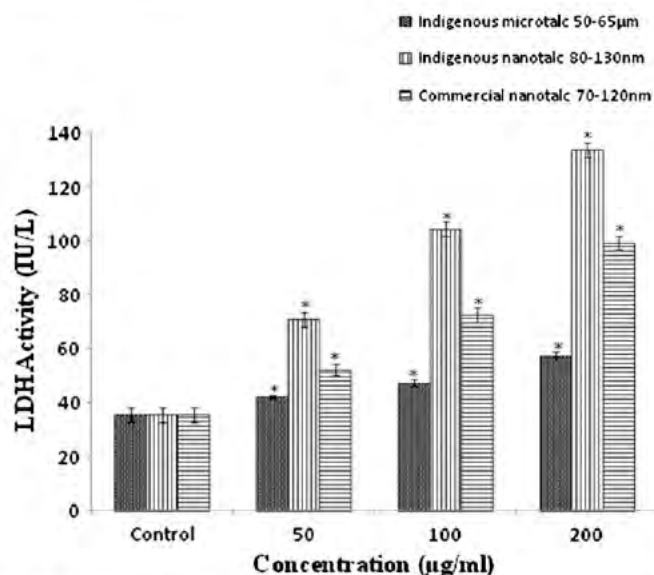


Fig. 3. The LDH activities in the cell culture medium after 48-h exposure to indigenous microtalc, indigenous nanotalc and commercial nanotalc particles at indicated concentrations. Values are mean \pm SD from three independent experiments. *Denotes a significant difference from the control ($p < 0.05$).

100, and 200 μg/ml, and evaluated to be 136%, 155%, and 175%, respectively for indigenous microtalc and 150%, 203%, and 265%, respectively for indigenous nanotalc and 136%, 175%, and 205%, respectively for commercial nanotalc (Fig. 4). The highest fluorescence obtained was that for indigenous nanotalc at 200 μg/ml.

3.6. Cellular GSH level and LPO induced by talc nanoparticles

Following exposure to talc particles at concentrations 50, 100, and 200 μg/ml for 48 h, the intracellular GSH level exhibited a concentration dependent decrease (Fig. 5). The GSH levels were reduced by 3%, 11.56%, and 18.8% for indigenous microtalc and 14.2%, 18.8%, and 25.4% for indigenous nanotalc and 6.6%, 11.5%, and 20.8%, respectively for commercial nanotalc.

In order to elucidate the lipid peroxidation induced by talc particles, the MDA concentration was measured. Each type of nanoparticles elevated the intracellular MDA concentration which was dependent on dosage and source of talc particle origins (Fig. 6). The MDA levels were elevated by 1.3 fold, 1.4 fold, and 1.9 fold, respectively for indigenous microtalc, and 1.6 fold, 2.3 fold, and 3.1 fold, respectively for indigenous nanotalc and 1.4 fold, 1.7 fold, and 2.1 fold, respectively for commercial nanotalc, compared to the control untreated groups.

3.7. Inhibitory effect afforded by ascorbic acid on LPO induced by talc nanoparticles

In an additional set of studies, L-ascorbic acid was added to the cells during exposure to micro and nanotalc, each group ex-

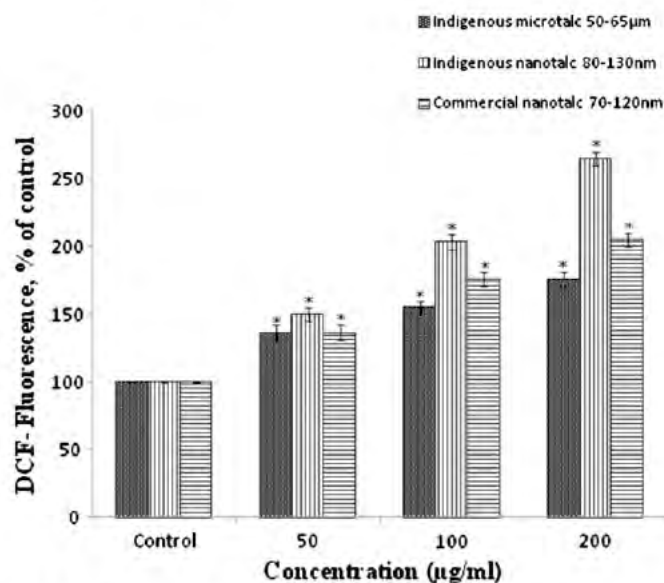


Fig. 4. DCF-fluorescence intensity after 48-h exposure to indigenous microtalc, indigenous nanotalc and commercial nanotalc particles at indicated concentrations. Values are mean \pm SD from three independent experiments. *Denotes a significant difference from the control ($p < 0.05$).

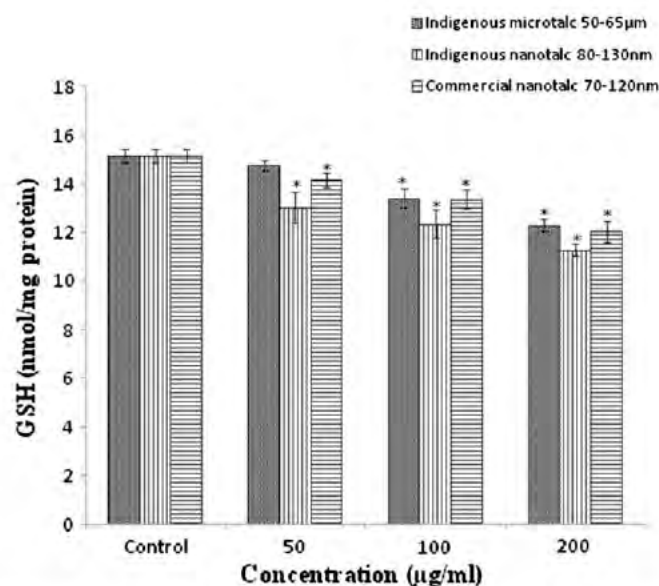


Fig. 5. Cellular GSH levels of A549 cells after 48-h exposure to indigenous microtalc, indigenous nanotalc and commercial nanotalc particles at indicated concentrations. Values are mean \pm SD from three independent experiments. *Denotes a significant difference from the control ($p < 0.05$).

posed at 200 μg/ml, as a test to determine if the oxidative damage to A549 cells could be prevented. Results show that L-ascorbic acid effectively prevented the generation of MDA level induced by talc particles (Fig. 7). MDA level was reduced up to control level for indigenous microtalc in the presence of ascorbic acid. When indigenous nanotalc induced MDA was 3.1 fold, in the presence of ascorbic acid it was reduced and found to be 2.1 fold of control. When commercial nanotalc induced MDA was 2.1 fold, in the presence of ascorbic acid it was 1.3 fold of control.

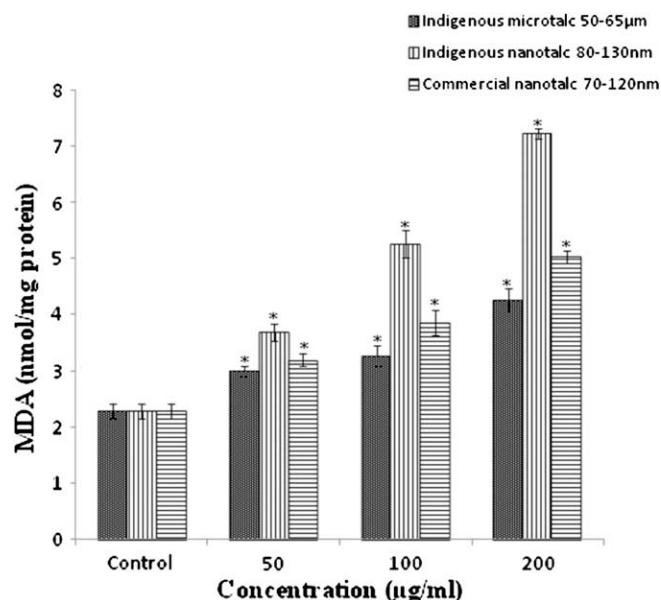


Fig. 6. Cellular MDA levels of A₅₄₉ cells after 48-h exposure to indigenous microtalc, indigenous nanotalc and commercial nanotalc particles at indicated concentrations. Values are mean \pm SD from three independent experiments. *Denotes a significant difference from the control ($p < 0.05$).

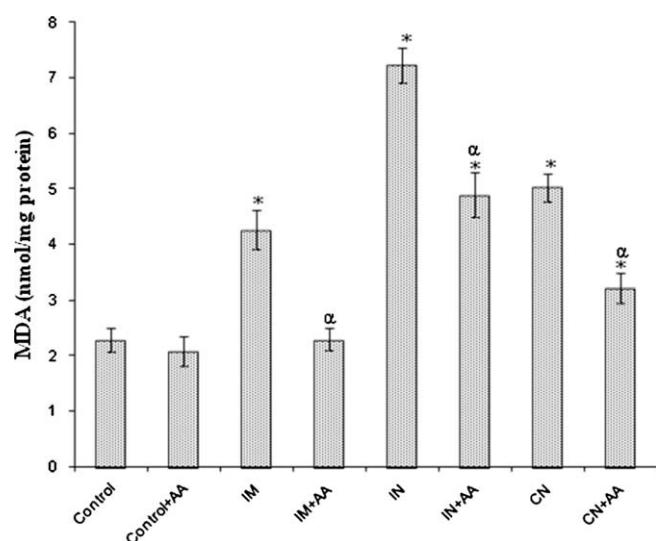


Fig. 7. Showing the inhibitory effect of ascorbic acid on cellular MDA levels of A₅₄₉ cells under indicated conditions of 48-h exposure. *AA (1.5 mM L-ascorbic acid); IM (200 µg/ml indigenous microtalc); IN (200 µg/ml indigenous nanotalc); CN (200 µg/ml commercial nanotalc). Values are mean \pm SD from three independent experiments. *Denotes a significant difference from the control ($p < 0.05$). α indicates the significant inhibitory effect of ascorbic acid (AA) on lipid peroxidation versus either, IM, IN or CN.

4. Discussion

At present, an *in vitro* toxicological study of talc nanoparticles is lacking. In this study, the cytotoxicity of two types of talc nanoparticles was investigated in cultured human bronchoalveolar carcinoma derived cells (A₅₄₉). This cell line has been widely used in *in vitro* cytotoxicity studies (Huang et al., 2004; Bakand et al., 2006). Present study showed that the two types of talc nanoparticles caused significant reduction in cell viability as a function of concentration and their iron content. The talc nanoparticles from two sources induced the enhanced generation of ROS and MDA

production. Consequently, redundant free radicals would interact with biomolecules including proteins, enzymes, membrane lipids and even DNA which could be oxidized, modified, destructured and ultimately dysfunctional (Marnett, 2000; Hensley and Floyd, 2002).

Oxidative stress has been suggested to play an important role in the mechanism of toxicity of a number of compounds whether by production of free radicals or by depleting cellular antioxidant capacity. Cellular integrity is affected by oxidative stress when the production of ROS overwhelms antioxidant defense mechanism (Halliwell et al., 1992; Chen and Yu, 1994). ROS are oxygen containing molecules, such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), and hydroxyl radical (HO[•]), that have a greater chemical activity than molecular oxygen. ROS are generated in many inflammatory conditions in the lung and have been associated with cell injury and apoptosis (Anderson et al., 1994; Meyer et al., 1993). Many other studies have shown that nanoparticles may produce toxicity by generating ROS. Recently, Buz'Zard and Lau (2007) have reported enhanced ROS generation in human ovarian cell culture and have found an increased cell proliferation and neoplastic transformation of human ovarian stromal and epithelial cells exposed with talc. In the present study too, talc micro and nanoparticles induced significantly higher ROS generation compared with untreated A₅₄₉ cells when using the fluorescent dichlorofluorescein probe. Moreover, indigenous nanotalc resulted higher ROS generation than commercial nanotalc.

GSH is the most abundant nonproteinous tripeptide containing a sulfhydryl group in virtually all cells, and it plays a significant role in many biological processes. It also constitutes the first line of the cellular defense mechanism against oxidative injury and is the major intracellular redox buffer in ubiquitous cell types (Meister, 1989). GSH acts as a cosubstrate in the GSH peroxidase catalyzed reduction of hydrogen peroxide or lipid peroxides (Forman et al., 1997) leading to its depletion. Previous studies demonstrated that ROS generation following GSH depletion caused mitochondrial damage (Martensson et al., 1989; Meister, 1995), which has been implicated in apoptosis (Green and Reed, 1998). There was a significant depletion of GSH between the control and the treated groups except for indigenous microtalc at 50 µg/ml. In terms of GSH depletion, indigenous nanotalc was found to be the most toxic.

In the toxicity mechanism of minerals, the iron content has been a key factor. Iron dependent ROS generation from fibers results in the generation of hydroxyl radicals through the Fenton reaction and the Haber Weiss cycle. Iron dependent LPO could be important, since this process requires redox cycling of iron and does not necessarily require H₂O₂ or ROS (Halliwell and Gutteridge, 1990). Indeed, iron has a key role in both the initiation and propagation of LPO, leading to the generation of peroxy and alkoxy radicals as well as lipid peroxides (Halliwell and Gutteridge, 1990). It has been known for several years that the surface iron (II) or leachable iron (II) on mineral surfaces reduces molecular oxygen to superoxide anion, which then dismutates to hydrogen peroxide. In the presence of asbestos or silica, hydrogen peroxide and superoxide react via a Fenton like reaction driven by iron to form the potent hydroxyl radical *in vitro* leading to cellular LPO (Mossman et al., 1996). Since, LPO is a sensitive parameter for toxic effects of various environmental pollutants with oxidative properties (Krug and Culig, 1991; Beck Speier et al., 2001; Oberdorster, 2004; Sayes et al., 2005); the authors suspected that the relatively high iron content in both the nanotalc may play a key role to yield higher ROS and in turn caused higher LPO. There are other nanomaterials, such as C₆₀, which mediates cytotoxicity primarily through lipid peroxidation (Sayes et al., 2005; Isakovic et al., 2006) whereas carboxyfullerenes (made by certain surface modifications of C₆₀) have been shown to impart cytoprotective activity by eliminating reactive oxygen species (ROS) and antago

nizing the effects of the oxidative stress dependent cytotoxicity (Dugan et al., 1997, 2001; Bogdanovic et al., 2004; Isakovic et al., 2006). Recently Scarfi et al. (2009) has reported that plasma membrane contact with quartz, a kind of silica, is sufficient to trigger membrane LPO, TNF α release and cell death in mouse macrophage cell line RAW 264.7. The authors hypothesize that contact of particles with cell membranes initiate ROS generation and LPO in a ratio of amount of iron present in talc nanoparticles.

For a given mass compared with larger particles, the ratio of surface to total atoms or molecules increases exponentially with decreasing particle size. Particle size is thereby an essential determinant of the fraction of reactive groups on particle surface (Oberdorster et al., 2005; Nel et al., 2006). For example, several studies found that ultrafine particles of titania are more toxic than its larger counterparts having the same chemical composition (Donaldson et al., 1998; Gilmour et al., 1997; Oberdorster et al., 1992, 1995; Oberdorster, 1996, 2000). Similarly, surface area dependent induction of oxidative stress and consequently, proinflammatory effects have been found to correlate in case of polystyrene particles by Brown et al. (2001) and Lin et al. (2006) have reported higher toxicity of the two sizes (15 and 46 nm) of silica nanoparticles than micro silica (5 μ m) on A₅₄₉ cells. Here two sizes (15 and 46 nm) of silica nanoparticles induced no significant differences in the toxicity and similar was the case in a study done by Sayes et al. (2006), where smaller nanoparticles of titania had effects comparable to larger nanoparticles of titania but showed a phase dependent differential toxicity where anatase titania (photoactive phase), able to generate ROS more strongly, was 100 times more toxic than an equivalent sample of rutile titania. In the present study, both nanoparticles would have resulted differential surface iron activity per given mass resulting in differential toxicity. When indigenous nanotalc induced toxicity is compared with indigenous microtalc, size dependent factor becomes apparent because all the compositional factors are constant. But when commercial nanotalc (having larger surface area but lower iron content) induced toxicity is compared with indigenous microtalc, the results show a complex function of size and impurities. Since, micro talc size is very large (50–65 μ m), than commercial nanotalc (70–120 nm), perhaps size becomes the primary determinant of toxicity, resulting in higher toxicity of commercial nanotalc than indigenous micro talc.

Another pathway of free radical generation by asbestos, silica or particulates like these (e.g. talc particles) occurs via an oxidative burst when fibers and particles are phagocytised by AMs or other cell types, including alveolar epithelial cells and fibroblasts (Churg, 1996). Phagocytic cells can endocytose small particles, whereas bigger crystals and fibers are subject to so called “frustrated phagocytosis”. Experimental studies suggest that in *in vivo* conditions “frustrated phagocytosis” appears to have a dramatic influence on the sustained generation of ROS (Hansen and Mossman, 1987; Vallyathan et al., 1992). Repeated “frustrated phagocytosis” would be expected to attract more phagocytes, resulting in chronic enhanced generation of ROS, which in turn contribute to inflammation, resulting in the secretion of IL 1 β leading to the initiation of pulmonary fibrosis (Dostert et al., 2008; Cassel et al., 2008). Since, talc and asbestos are physically and chemically similar, found together in nature and being particulate structure like silica and asbestos, talc particles may also generate ROS through activation of NADPH oxidase by frustrated phagocytosis, leading to the initiation of so called talcosis particularly in occupationally exposed workers.

Antioxidants, such as α tocopherol, uric acid and L ascorbic acid, typically prevent cellular damages caused by oxygen radicals by acting as ROS scavengers (Packer et al., 1979; Burton and Ingold, 1981). Ascorbic acid (or vitamin C) acts as a potent water soluble antioxidant in biological fluids (Frei et al., 1989, 1990) by scavenging physiologically relevant ROS and reactive nitrogen species

(RNS) (Halliwell, 1996). However, it should be noted that antioxidant potential of ascorbic acid has not been validated in certain conditions (Bowry et al., 1992; Poulsen et al., 1998; Levine et al., 1998). Ascorbic acid contributes significantly to cellular antioxidant activity as a water soluble chain breaking radical scavenger (Asard, 2008) and to the recycling of plasma membrane α tocopherol (vitamin E) via the reduction of the α tocopheroxyl radical (Aguirre and May, 2008). The latter activity may assist ascorbic acid to protect against LPO in membranes (May et al., 1998). We, therefore, tested the LPO preventive potential of antioxidant L ascorbic acid, on nanotalc and microtalc challenged A₅₄₉ cells. Results show that 1.5 mM L ascorbic acid effectively, but not completely, inhibited MDA level induced by talc nanoparticles. Determining the optimum concentration of ascorbic acid that might completely suppress LPO without causing any side effect is a matter of concern (Halliwell, 1999) and the evaluation of interrelationship between LPO and chelating effect of iron present on the surface of talc particles by deferoxamine mesylate on LPO is under investigation. Oxidative stress is known to elicit varying effects on the activity of antioxidant enzymes. The three primary scavenger enzymes involved in detoxifying ROS in mammalian systems are catalase, superoxide dismutase and glutathione peroxidase (Matés et al., 1999). For example the activity of GPx can provide important clue about the consumption rate of GSH in enzymatic detoxification of ROS. The activity of antioxidant enzymes can therefore provide further insight in understanding the mechanism of toxicity caused by talc particles and is currently under investigation.

5. Conclusion

We have presented a preliminary data on the toxicity response elicited by the two types of talc nanoparticles, depending on their different geological origin. Since, talc with a multitude of physical and functional characteristics due to different geological context and deposits, is used for particular applications, so occupational and consumer exposures to talc and its toxic effects are likely to vary accordingly, which is obvious in this study. The cytotoxicity seems to be due to primarily through induction of LPO, as a potential mechanism of toxicity discussed above. Addition of 1.5 mM of L ascorbic acid, a ROS scavenger, significantly, though not completely, reduced LPO. Data clearly suggest that exposure of talc, particularly nanopowder, should be protected in humans at risk of occupational as well as domestic exposure.

Acknowledgments

One of the authors (MJA) gratefully acknowledges the financial support provided by University Grants Commission (UGC), Govt. of India and CSIR networked project (NWP 17). The authors thank Dr. Alok Dhawan (*In Vitro* Toxicology Division, IITR, Lucknow, India) for providing DLS measurements. The IITR Communication no. of this article is 2782.

References

- Aguirre, R., May, J.M., 2008. Inflammation in the vascular bed: importance of vitamin C. *Pharmacology and Therapeutics* 119, 96–103.
- Anderson, M.T., Staal, F.J., Gitler, C., Herzenberg, L.A., 1994. Separation of oxidant-initiated and redox-regulated steps in the NF- κ B signal transduction pathway. *Proceedings of the National Academy of Sciences USA* 91, 11527–11531.
- Asard, H., 2008. Ascorbate. In: Banerjee, R. (Ed.), *Redox Biochemistry*. A John Wiley and Sons Inc., Hoboken, pp. 22–27.
- Bakand, S., Winder, C., Khalil, C., Hayes, A., 2006. A novel *in vitro* exposure technique for toxicity testing of selected volatile organic compounds. *Journal of Environmental Monitoring* 8, 100–105.
- Beck-Speier, I., Dayal, N., Karg, E., Maier, K.L., Roth, C., Ziesenis, A., Heyder, J., 2001. Agglomerates of ultrafine particles of elemental carbon and TiO₂ induce generation of lipid mediators in alveolar macrophages. *Environmental Health Perspectives* 109, 613–618.

- Bogdanovic, G., Kojic, V., Dordevic, A., Canadanovic-Brunet, J., Vojinovic-Miloradov, M., Baltic, V.V., 2004. Modulating activity of fullerol C-60 (OH)₍₂₂₎ on doxorubicin-induced cytotoxicity. *Toxicology in Vitro* 18, 629–637.
- Borm, P.J., Robbins, D., Haubold, S., Kuhlbusch, T., Fissan, H., Donaldson, K., Schins, R., Stone, V., Kreyling, W., Lademann, J., 2006. The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and Fibre Toxicology* 3, 11.
- Bowry, V.W., Ingold, K.U., Stocker, R., 1992. Vitamin-E in human low density-lipoprotein—when and how this antioxidant becomes a prooxidant. *Biochemical Journal* 4, 288–341.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248–254.
- Brown, D.M., Wilson, M.R., MacNee, W., Stone, V., Donaldson, K., 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology and Applied Pharmacology* 175, 191–199.
- Burton, G.W., Ingold, K.U., 1981. Autoxidation of biological molecules. 1. The antioxidant activity of vitamin-E and related chain-breaking phenolic antioxidants in vitro. *Journal of the American Chemical Society* 103, 6472–6477.
- BuzZard, A.R., Lau, B.H., 2007. Pycnogenol reduces talc-induced neoplastic transformation in human ovarian cell cultures. *Phytotherapy Research* 21, 579–586.
- Cassel, S.L., Eisenbarth, S.C., Iyer, S.S., Sadler, J.J., Colegio, O.R., Tephly, L.A., Carter, A.B., Rothman, P.B., Flavell, R.A., Sutterwala, F.S., 2008. The Nalp3 inflammasome is essential for the development of silicosis. *Proceedings of the National Academy of Sciences USA* 105, 9035–9040.
- Chang, S., Risch, H.A., 1997. Perineal talc exposure and risk of ovarian carcinoma. *Cancer* 79, 2396–2401.
- Chen, J.J., Yu, B.P., 1994. Alteration in mitochondrial membrane fluidity by lipid peroxidation products. *Free Radical Biology and Medicine* 17, 411–418.
- Churg, A., 1996. The uptake of mineral particles by pulmonary epithelial cells. *American Journal of Respiratory and Critical Care Medicine* 154, 1124–1140.
- Cook, L.S., Kamb, M.L., Weiss, N.S., 1997. Perineal powder exposure and the risk of ovarian cancer. *American Journal of Epidemiology* 145, 459–465.
- Cramer, D.W., Liberman, R.E., Titus-Ernstoff, L., Welch, W.R., Greenberg, E.R., Baron, J.A., Harlow, B.L., 1999. Genital talc exposure and risk of ovarian cancer. *International Journal of Cancer* 81, 351–356.
- Croue, F., Gaubin, Y., Prevost, M.C., Beaupain, R., Pianezzi, B., Soleihavoup, J.P., 1990. Effects of hypergravity on lung carcinoma cells maintained in continuous organotypic culture. *Aviation, Space, and Environmental Medicine*, 1002–1006.
- Donaldson, K., Li, X.Y., MacNee, W., 1998. Ultrafine (nanometre) particle mediated lung injury. *Journal of Aerosol Science* 29, 553–560.
- Donaldson, K., Brown, D., Clouter, A., Duffin, R., MacNee, W., Renwick, L., Tran, L., Stone, V., 2002. The pulmonary toxicology of ultrafine particles. *Journal of Aerosol Medicine* 15, 213–220.
- Dostert, C., Pétrilli, V., Bruggen, R.V., Steele, C., Mossman, B.T., Tschopp, J., 2008. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–677.
- Dugan, L.L., Lovett, E.G., Quick, K.L., Lotharius, J., Lin, T.T., O'Malley, K.L., 2001. Fullerene-based antioxidants and neurodegenerative disorders. *Parkinsonism and Related Disorders* 7, 243–246.
- Dugan, L.L., Turetsky, D.M., Du, C., Lobner, D., Wheeler, M., Almli, C.R., Clifton, K.F., Shen, C.K.F., Luh, T.Y., Choi, D.W., Lin, T.S., 1997. Carboxyfullerenes as neuroprotective agents. *Proceedings of the National Academy of Sciences USA* 94, 9434–9439.
- Forman, H.J., Liu, R., Tian, L., 1997. Glutathione cycling in oxidative stress. In: Clerch, L.B., Massaro, D.J. (Eds.), *Oxygen, Gene Expression, and Cellular Function: Lung Biology in Health and Disease*, vol. 105. Marcel Dekker, New York, pp. 99–112.
- Frei, B., England, L., Ames, B.N., 1989. Ascorbate is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences USA* 86, 6377–6381.
- Frei, B., Stocker, R., England, L., Ames, B.N., 1990. Ascorbate: the most effective antioxidant in human blood plasma. *Advances in Experimental Medicine and Biology* 264, 155–163.
- Gates, M.A., Tworoger, S.S., Terry, K.L., Titus-Ernstoff, L., Rosner, B., Vivo, I.D., Cramer, D.W., Hankinson, S.E., 2008. Talc use, variants of the *GSTM1*, *GSTT1*, and *NAT2* genes, and risk of epithelial ovarian cancer. *Cancer Epidemiology, Biomarkers and Prevention* 17, 2436–2444.
- Gilmour, P., Brown, D.M., Beswick, P.H., Benton, E., MacNee, W., Donaldson, K., 1997. Surface free radical activity of PM10 and ultrafine titanium dioxide: A unifying factor in their toxicity? *The Annals of Occupational Hygiene* 41 (Suppl. 1), 32–38.
- Green, D.G., Reed, J.C., 1998. Mitochondria and apoptosis. *Science* 281, 1309–1312.
- Halliwell, B., Gutteridge, J.M., 1990. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology* 186, 1–85.
- Halliwell, B., 1996. Vitamin C: antioxidant or pro-oxidant in vivo? *Free Radical Research* 25, 439–454.
- Halliwell, B., 1999. Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *Nutrition Reviews* 57, 104–113.
- Halliwell, B., Gutteridge, J.M., Cross, C.E., 1992. Free radicals, antioxidants, and human disease: where are we now? *The Journal of Laboratory and Clinical Medicine* 119, 598–620.
- Hansen, K., Mossman, B.T., 1987. Generation of superoxide (O²⁻) from alveolar macrophages exposed to asbestiform and non-asbestiform particles. *Cancer Research* 47, 1681–1686.
- Hansen, M.B., Nielsen, S.E., Berg, K., 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/kill. *Journal of Immunological Methods* 119, 203–210.
- Harlow, B.L., Cramer, D.W., Bell, D.A., Welch, W.R., 1992. Perineal exposure to talc and ovarian cancer risk. *Obstetrics and Gynecology* 80, 19–26.
- Harlow, B.L., Hartge, P.A., 1995. A review of perineal talc exposure and risk of ovarian cancer. *Regulatory Toxicology and Pharmacology* 21, 254–260.
- Heller, D.S., Westhoff, C., Gordon, R.E., Katz, N., 1996. The relationship between perineal cosmetic talc usage and ovarian talc particle burden. *American Journal of Obstetrics and Gynecology* 174, 1507–1510.
- Henderson, W.J., Joslin, C.A., Turnbull, A.C., Griffiths, K., 1971. Talc and carcinoma of the ovary and cervix. *The Journal of Obstetrics and Gynaecology of the British Commonwealth* 78, 266–272.
- Hensley, K., Floyd, R.A., 2002. Reactive oxygen species and protein oxidation in aging: a look back, a look ahead. *Archives of Biochemistry and Biophysics* 397, 377–383.
- Hissin, P.J., Hilf, R., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Analytical Biochemistry* 74, 214–226.
- Hollinger, M.A., 1990. Pulmonary toxicity of inhaled and intravenous talc. *Toxicology Letters* 52, 121–127.
- Holsapple, M.P., Farland, W.H., Landry, T.D., Monteiro-Riviere, N.A., Carter, J.M., Walker, N.J., Thomas, K.V., 2005. Research strategies for safety evaluation of nanomaterials, part II: toxicological and safety evaluation of nanomaterials, current challenges and data needs. *Toxicological Sciences* 88, 12–17.
- Huang, M., Khor, E., Lim, L.Y., 2004. Uptake and cytotoxicity of chitosan molecules and nanoparticles: effects of molecular weight and degree of deacetylation. *Pharmaceutical Research* 21, 344–353.
- Isakovic, A., Markovic, Z., Todorovic-Markovic, B., Nikolic, N., Vranjes-Djuric, S., Mirkovic, M., Dramicanin, M., Harhaji, L., Raicevic, N., Nikolic, Z., Trajkovic, V., 2006. Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene. *Toxicological Sciences* 91, 173–183.
- Kipen, H.M., Laskin, D.L., 2005. Smaller is not always better: nanotechnology yields nanotoxicology. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 289, L696–L697.
- Krug, H.F., Culig, H., 1991. Directed shift of fatty-acids from phospholipids to triacylglycerols in HL-60 cells induced by nanomolar concentrations of triethyl lead chloride – involvement of a pertussis toxin-sensitive pathway. *Molecular Pharmacology* 39, 511–516.
- Levine, M.A., Daruwala, R.C., Park, J.B., Rumsey, S.C., Wang, Y., 1998. Does vitamin C have a pro-oxidant effect? *Nature (London)* 395, 231.
- Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W., Todor, G., 1976. A continuous tumor cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *International Journal of Cancer* 17, 62–70.
- Lin, W., Huang, Y.W., Zhou, X.D., Ma, Y., 2006. In vitro toxicity of silica nanoparticles in human lung cancer cells. *Toxicology and Applied Pharmacology* 217, 252–259.
- Marnett, L.J., 2000. Oxyradicals and DNA damage. *Carcinogenesis* 21, 361–370.
- Martensson, J., Jain, A., Frayer, W., Meister, A., 1989. Glutathione metabolism in the lung: inhibition of its synthesis leads to lamellar body and mitochondrial defects. *Proceedings of the National Academy of Sciences USA* 86, 5296–5300.
- Matés, J.M., Pérez-Gómez, C., DeCastro, I.N., 1999. Antioxidant enzymes and human diseases. *Clinical Biochemistry* 32, 595–603.
- May, J.M., Qu, Z.-C., Mendiratta, S., 1998. Protection and recycling of α -tocopherol in human erythrocytes by intracellular ascorbic acid. *Archives of Biochemistry and Biophysics* 349, 281–289.
- Meister, A., 1989. Molecular properties and clinical applications. In: *Glutathione Centennial*. Academic Press, New York.
- Meister, A., 1995. Mitochondrial changes associated with glutathione deficiency. *Biochimica et Biophysica Acta* 1271, 35–42.
- Meyer, M., Schreck, R., Baeuerle, P.A., 1993. H₂O₂ and antioxidants have opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *The EMBO Journal* 12, 2005–2015.
- Mills, P.K., Riordan, D.G., Cress, R.D., Young, H.A., 2004. Perineal talc exposure and epithelial ovarian cancer risk in the Central Valley of California. *International Journal of Cancer* 112, 458–464.
- Mossman, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65, 55–63.
- Mossman, B.T., Kamp, D.W., Weitzman, S.A., 1996. Mechanisms of carcinogenesis and clinical features of asbestos-associated cancers. *Cancer Investigation* 14, 466–480.
- National Toxicology Program, 1993. *NTP Toxicology and Carcinogenesis Studies of Talc (Non-asbestiform) in Rats and Mice (Inhalation Studies)*, vol. 421, pp. 1–287.
- Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. *Science* 311, 622–627.
- Oberdorster, G., Ferin, J., Gelein, R., Soderholm, S., Finkelstein, J., 1992. Role of alveolar macrophage in lung injury: studies with ultrafine particles. *Environmental Health Perspectives* 97, 193–199.
- Oberdorster, G., Gelein, R., Ferin, J., Weiss, B., 1995. Association of particulate air pollution and acute mortality: involvement of ultrafine particles? *Inhalation Toxicology* 7, 111–124.
- Oberdorster, G., 1996. Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles. *Inhalation Toxicology* 8, 73–89.
- Oberdorster, G., 2000. Toxicology of ultrafine particles: in vivo studies. *Philosophical Transactions of the Royal Society* 358, 2719–2740.

- Oberdorster, E., 2004. Manufactured nanomaterials (fullerenes, C-60) induce oxidative stress in the brain of juvenile largemouth bass. *Environmental Health Perspective* 112, 1058–1062.
- Oberdorster, G., Oberdorster, E., Oberdorster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspective* 113, 823–839.
- Ohkawa, H., Ohisi, N., Yagi, Y., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95, 351–358.
- Packer, J.E., Slater, T.F., Willson, R.L., 1979. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature (London)* 278, 737–738.
- Poulsen, H.E., Weimann, A., Salonen, J.T., Nyyssonen, K., Loft, S., Cadet, J., Douki, T., Ravanat, J., 1998. Does vitamin C have a pro-oxidant effect? *Nature (London)* 395, 231–232.
- Sayes, C.M., Gobin, A.M., Ausman, K.D., Mendez, J., West, J.L., Colvin, V.L., 2005. Nano-C (60) cytotoxicity is due to lipid peroxidation. *Biomaterials* 26, 7587–7595.
- Sayes, C.M., Wahi, R., Kurian, P.A., Liu, Y., West, J.L., Ausman, K.D., Warheit, D.B., Colvin, V.L., 2006. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. *Toxicological Sciences* 92, 174–185.
- Scarfi, S., Magnone, M., Ferraris, C., Pozzolini, M., Benvenuto, F., Benatti, U., Giovine, M., 2009. Ascorbic acid pre-treated quartz stimulates TNF- α release in RAW 264.7 murine macrophages through ROS production and membrane lipid peroxidation. *Respiratory Research* 10, 25.
- Vallyathan, V., Mega, J.F., Shi, X., Dalal, N.S., 1992. Enhanced generation of free radicals from phagocytes induced by mineral dusts. *American Journal of Respiratory Cell and Molecular Biology* 6, 404–413.
- Wang, H., Joseph, J.A., 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biology and Medicine* 27, 612–616.
- Welder, A.A., Grant, R., Bradlaw, J., Acosta, D., 1991. A primary culture system of adult rat heart cells for the study of toxicologic agent. *In Vitro Cellular and Developmental Biology* 27A, 921–926.
- Wild, P., 2006. Lung cancer risk and talc not containing asbestiform fibres: a review of the epidemiological evidence. *Occupational and Environmental Medicine* 63, 4–9.
- Werebe, E.C., Pazetti, R., 1999. Systemic distribution of talc after intrapleural administration in rats. *Chest* 115, 190–193.
- Wroblewski, F., LaDue, J.S., 1955. Lactate dehydrogenase activity in blood. *Proceedings of the Society for Experimental Biology and Medicine* 90, 210–213.

Exhibit 96

Molecular Basis Supporting the Association of Talcum Powder Use With Increased Risk of Ovarian Cancer

Reproductive Sciences
1-10

© The Author(s) 2019

Article reuse guidelines:

sagepub.com/journals-permissions

DOI: 10.1177/1933719119831773

journals.sagepub.com/home/rsx



Nicole M. Fletcher, PhD¹, Amy K. Harper, MD², Ira Memaj, BS¹,
Rong Fan, MS¹, Robert T. Morris, MD², and Ghassan M. Saed, PhD^{1,2}

Abstract

Genital use of talcum powder and its associated risk of ovarian cancer is an important controversial topic. Epithelial ovarian cancer (EOC) cells are known to manifest a persistent prooxidant state. Here we demonstrated that talc induces significant changes in key redox enzymes and enhances the prooxidant state in normal and EOC cells. Using real-time reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assay, levels of CA-125, caspase-3, nitrate/nitrite, and selected key redox enzymes, including myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GSR), were determined. TaqMan genotype analysis utilizing the QuantStudio 12K Flex was used to assess single-nucleotide polymorphisms in genes corresponding to target enzymes. Cell proliferation was determined by MTT proliferation assay. In all talc-treated cells, there was a significant dose-dependent increase in prooxidant iNOS, nitrate/nitrite, and MPO with a concomitant decrease in antioxidants CAT, SOD, GSR, and GPX ($P < .05$). Remarkably, talc exposure induced specific point mutations that are known to alter the activity in some of these key enzymes. Talc exposure also resulted in a significant increase in inflammation as determined by increased tumor marker CA-125 ($P < .05$). More importantly, talc exposure significantly induced cell proliferation and decreased apoptosis in cancer cells and to a greater degree in normal cells ($P < .05$). These findings are the first to confirm the cellular effect of talc and provide a molecular mechanism to previous reports linking genital use to increased ovarian cancer risk.

Keywords

talc, epithelial ovarian cancer, oxidative stress, single-nucleotide polymorphism, cell proliferation

Introduction

Ovarian cancer is the most lethal gynecologic malignancy and ranks fifth in cancer deaths among women diagnosed with cancer.¹ Epithelial ovarian cancer (EOC) has long been considered a heterogeneous disease with respect to histopathology, molecular biology, and clinical outcome.^{1,2} Although surgical techniques and treatments have advanced over the years, the prognosis of EOC remains poor, with a 5-year survival rate of 50% in advanced stage.² This is largely due to the lack of early warning symptoms and screening methods and the development of chemoresistance.^{1,2} Moreover, ovarian cancer is known to be associated with germline mutations in the *BRCA1* or *BRCA2* genes, but with a rate of only 20 % to 40%, suggesting the presence of other unknown mutations in other predisposition genes.³ Additional genetic variations including single-nucleotide polymorphisms (SNPs) have been hypothesized to act as low to moderate penetrant alleles that contribute to ovarian cancer risk.^{3,4}

The pathophysiology of EOC is not fully understood but has been strongly associated with inflammation and the resultant

oxidative stress.⁵ We have previously characterized EOC cells to manifest a persistent prooxidant state as evident by the upregulation of key oxidants and downregulation of key antioxidants, which is further enhanced in chemoresistant EOC cells.⁶ The expression of key prooxidant/inflammatory enzymes such as inducible nitric oxide synthase (iNOS), nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, and myeloperoxidase (MPO), as well as an increase in nitric oxide (NO) levels, was increased in EOC tissues and cells.⁶ Additionally, we have shown that EOC cells manifest lower apoptosis, which

¹ Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA

² Department of Gynecologic Oncology, Karmanos Cancer Institute, Detroit, MI, USA

Corresponding Author:

Ghassan M. Saed, Departments of Obstetrics and Gynecology and Oncology, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA.

Email: gsaed@med.wayne.edu

was markedly induced by inhibiting iNOS, indicating a strong link between apoptosis and NO/iNOS pathways in these cells.⁶

The cellular redox balance is maintained by key antioxidants including catalase (CAT), superoxide dismutase (SOD), or by glutathione peroxidase (GPX) coupled with glutathione reductase (GSR).⁵ Other important scavengers include thioredoxin coupled with thioredoxin reductase, and glutaredoxin, which utilizes glutathione (GSH) as a substrate.⁷ We have previously reported that a genotype switch in key antioxidants is a potential mechanism leading to the acquisition of chemoresistance in EOC cells.⁷ We have studied the effects of genetic polymorphisms in key redox genes on the acquisition of the oncogenic phenotype in EOC cells, including genes that control the levels of cellular reactive oxygen species and oxidative damage and SNPs for genes involved in carcinogen metabolism (detoxification and/or activation), antioxidants, and DNA repair pathways.^{4,6} Several function-altering SNPs have been identified in key antioxidants, including CAT, GPX, GSR, and SOD.⁴

Several studies have suggested the possible association between genital use of talcum powder and risk of EOC.⁷⁻¹² Association between the use of cosmetic talc in genital hygiene and ovarian cancer was first described in 1982 by Cramer et al, and many subsequent studies supported this finding.⁷⁻¹² Talc and asbestos are both silicate minerals; the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature.⁷⁻¹² Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response.⁷ The objective of this study was to determine the effects of talcum powder on the expression of key redox enzymes, CA-125 levels, and cell proliferation and apoptosis in normal and EOC cells.

Material and Methods

Cell Lines

Ovarian cancer cells SKOV-3 (ATCC), A2780 (Sigma Aldrich, St Louis, Missouri), and TOV112D (a kind gift from Gen Sheng Wu at Wayne State University, Detroit, Michigan) and normal cells human macrophages (EL-1; ATCC, Manassas, Virginia), human primary normal ovarian epithelial cells (Cell Biologics, Chicago, Illinois), human ovarian epithelial cells (HOSEpiC; ScienCell Research Laboratories, Inc, Carlsbad, California), and immortalized human fallopian tube secretory epithelial cells (FT33; Applied Biological Materials, Richmond, British Columbia, Canada) were used. All cells were grown in media and conditions following manufacturer's protocol. EL-1 cells were grown in IMDM media (ATCC) supplemented with 0.1 mM hypoxanthine and 0.1 mM thymidine solution (H-T, ATCC) and 0.05 mM β -mercaptoethanol. SKOV-3 EOC cells were grown in HyClone McCoy's 5A medium (Fisher Scientific, Waltham, Massachusetts), A2780 EOC cells were grown in HyClone RPMI-1640 (Fisher Scientific), and both TOV112D EOC cells were grown in MCDB105

(Cell Applications, San Diego, California) and Medium 199 (Fisher Scientific; 1:1). All media were supplemented with fetal bovine serum (Innovative Research, Novi, Michigan) and penicillin/streptomycin (Fisher Scientific), per their manufacturer specifications. Human primary normal ovarian epithelial cells were grown in complete human epithelial cell medium (Cell Biologics).

Treatment of Cells

Talcum baby powder (Johnson & Johnson, New Brunswick, NJ, #30027477, Lot#13717RA) was dissolved in dimethyl sulfoxide (DMSO; Sigma Aldrich) at a concentration of 500 mg in 10 mL and was filtered with a 0.2 μ m syringe filter (Corning). Sterile DMSO was used as a control for all treatments. Cells were seeded in 100-mm cell culture dishes (3×10^6) and were treated 24 hours later with 5, 20, or 100 μ g/mL of talc for 72 hours. Cell pellets were collected for RNA, DNA, and protein extraction. Cell culture media were collected for CA-125 analysis by enzyme-linked immunosorbent assay (ELISA).

Real-Time Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from all cells using the RNeasy mini kit (Qiagen, Valencia, California). Measurement of the amount of RNA in each sample was performed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). A 20 μ L complementary DNA reaction volume containing 0.5 μ g RNA was prepared using the SuperScript VILO Master Mix Kit (Life Technologies, Carlsbad, California). Optimal oligonucleotide primer pairs were selected for each target using Beacon designer (Premier Biosoft, Inc; Table 1). Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using the EXPRESS SYBR GreenER qPCR supermix kit (Life Technologies) and the Cepheid 1.2f detection system (Sunnyvale, CA) previously described.⁶ Standards with known concentrations and lengths were designed specifically for β -actin (79 bp), CAT (105 bp), NOS2 (89 bp), GSR (103 bp), GPX1 (100 bp), MPO (79 bp), and SOD3 (84 bp), allowing for construction of a standard curve using a 10-fold dilution series.⁶ All samples were normalized to β -actin. A final melting curve analysis was performed to demonstrate specificity of the PCR product.

Protein Detection

Cell pellets were lysed utilizing cell lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton, 2.5 sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, 1 μ g/mL leupeptin) containing a cocktail of protease inhibitors. Samples were centrifuged at 13 000 rpm for 10 minutes at 4°C. Total protein concentration of cell lysates from control and talc-treated cells was measured with the Pierce BCA protein assay kit (Thermo Scientific, Rockford, Illinois).

Table 1. Real Time RT PCR Oligonucleotide Primers.

Accession Number	Gene	Sense (5' 3')	Antisense (3' 5')	Amplicon (bp)	Annealing Time (seconds) and Temperature (°C)
NM 001101	β actin	ATGACTTAGTTGCGTTACAC	AATAAAGCCATGCCAATCTC	79	10, 64
NM 001752	CAT	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCAGGATAG	105	10, 63
NM 003102	SOD3	GTGTTCTGCTGCTCCT	TCCGCCGAGTCAGAGTTG	84	60, 64
NM 000637	GSR	TCACCAAGTCCCATATAGAAATC	TGTGGCGATCAGGATGTG	116	10, 63
NM 000581	GPX1	GGACTACACCCAGATGAAC	GAGCCCTTGCGAGGTGTAG	91	10, 66
NM 000625	NOS2	GAGGACCACATCTACCAAGGAGGAG	CCAGGCAGGCGGAATAGG	89	30, 59
NM 000250	MPO	CACTTGTATCCTCTGGTTCTTCAT	TCTATATGCTTCTCACGCCTAGTA	79	60, 63

Abbreviation: RT-PCR, reverse transcription polymerase chain reaction.

Detection of Protein/Activity by ELISA

The following ELISA kits were used (Cayman Chemical, Ann Arbor, Michigan): CAT, SOD, GSR, GPX, and MPO. Nitrite (NO_2^-)/nitrate (NO_3^-) were determined spectrophotometrically by Griess assay as previously reported.⁶ CA-125 protein levels were measured in cell media by ELISA (Ray Biotech, Norcross, Georgia).

TaqMan SNP Genotyping Assay

DNA was isolated utilizing the EZ1 DNA tissue kit (Qiagen) for EOC cells. The TaqMan SNP genotyping assay set (Applied Biosystems, Carlsbad, California; NCBI dbSNP genome build 37, MAF source 1000 genomes) was used to genotype the SNPs (Table 1). The Applied Genomics Technology Center (AGTC, Wayne State University) performed these assays. Analysis was done utilizing the QuantStudio 12 K Flex real-time PCR system (Applied Biosystems).

Cell Proliferation and Apoptosis

Cell proliferation was assessed with the TACS MTT cell proliferation assay (Trevigen, Gaithersburg, Maryland) after treatment with talc (100 $\mu\text{g/mL}$) for 24 hours. The Caspase-3 Colorimetric Activity Assay Kit (Chemicon, Temecula, California) was used to determine levels of caspase-3 activity after treatment of normal and EOC cells with various doses of talc as previously described.⁶ Equal concentrations of cell lysate were used. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate DEVD-pNA. The free pNA can be quantified using a spectrophotometer or a microtiter plate reader at 405 nm. Comparison of the absorbance of pNA from an apoptotic sample with its control allows determination of the percentage increase in caspase-3 activity.

Statistical Analysis

Normality was examined using the Kolmogorov-Smirnov test and by visual inspection of quantile-quantile plots. Because most of the data were not normally distributed, differences in distributions were examined using the Kruskal-Wallis test.

Generalized linear models were fit to examine pairwise differences in estimated least squares mean expression values by exposure to 0, 5, 20, or 100 $\mu\text{g/mL}$ of talc. We used the Tukey-Kramer adjustment for multiple comparisons, and the regression models were fit using log2 transformed analyte expression values after adding a numeric constant "1" to meet model assumptions while avoiding negative transformed values. *P* values below .05 are statistically significant.

Results

Talc Treatment Decreased the Expression of Antioxidant Enzymes SOD and CAT in Normal and EOC Cells

Real-time RT-PCR and ELISA assays were utilized to determine the CAT and SOD messenger RNA (mRNA) and protein levels in cells before and after 72 hours talc treatment, respectively (Figure 1). The CAT (Figure 1A and C) and SOD (Figure 1B and D) mRNA and protein levels were significantly decreased in a dose-dependent manner in talc-treated cells compared to controls ($P < .05$).

Talc Treatment Increased the Expression of Prooxidants iNOS, NO_2^- / NO_3^- , and MPO in Normal and EOC Cells

Real-time RT-PCR and NO_2^- / NO_3^- assays were utilized to determine the iNOS mRNA and NO levels in cells before and after 72 hours talc treatment, respectively (Figure 2). The iNOS mRNA and NO levels were significantly increased in a dose-dependent manner in talc-treated cells as compared to their controls (Figure 2A and C, $P < .05$). As expected, there was no detectable MPO in normal ovarian and fallopian tube cells, and thus, talc treatment did not have any effect. However, MPO mRNA and protein levels were significantly increased in a dose-dependent manner in talc-treated ovarian cancer cells and macrophages compared to controls (Figure 2B and D, $P < .05$).

Talc Treatment Decreased the Expression of Antioxidant Enzymes, GPX and GSR, in Normal and EOC Cells

Real-time RT-PCR and ELISA assays were utilized to determine the GPX and GSR mRNA and protein levels in cells before and

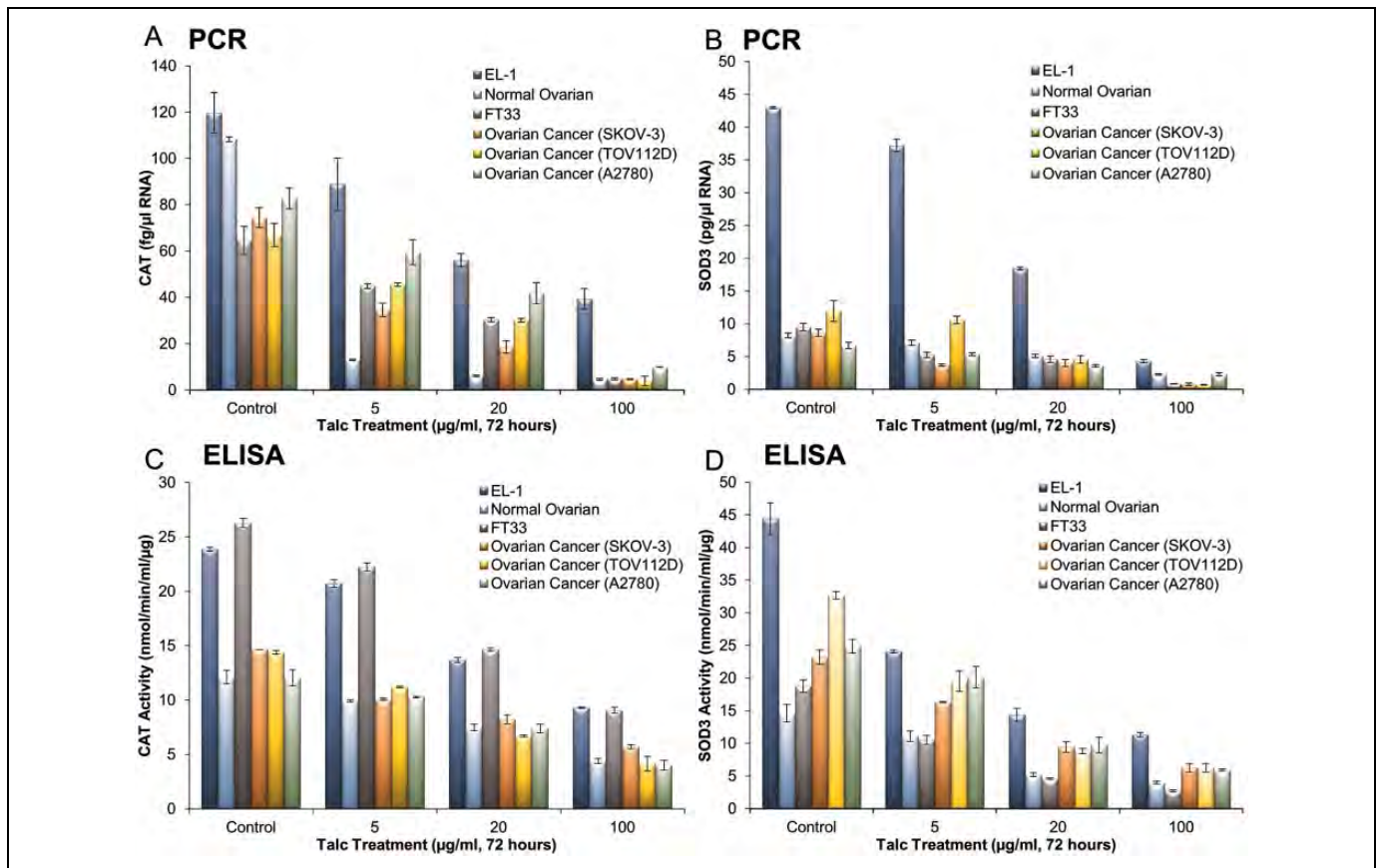


Figure 1. Decreased expression and activity of key antioxidant enzymes, CAT and SOD3. The mRNA (real time RT PCR) and protein/activity levels (ELISA) of CAT (A and C) and SOD3 (B and D) were determined in macrophages (EL 1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV 3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells and in all doses as compared to controls. CAT indicates catalase; SOD3, superoxide dismutase 3; mRNA, messenger RNA; RT PCR, reverse transcription polymerase chain reaction; ELISA, enzyme linked immunosorbent assay.

after 72 hours of talc treatment, respectively (Figure 3). The GPX (Figure 3A and C) and GSR (Figure 3B and D) mRNA and protein levels were significantly decreased in a dose-dependent manner in talc-treated cells compared to controls ($P < .05$).

Talc Exposure Induced Known Genotype Switches in Key Oxidant and Antioxidant Enzymes

Talc treatment was associated with a genotype switch in *NOS2* from the common C/C genotype in untreated cells to T/T, the SNP genotype, in talc-treated cells, except in A2780 and TOV112D (Table 2). Additionally, the observed decrease in CAT expression and activity was associated with a genotype switch from common C/C genotype in CAT in untreated cells to C/T, the SNP genotype, in TOV112D and all normal talc-treated cells. However, there was no detectable genotype switch in CAT in A2780, SKOV3, and TOV112D (Table 2). Remarkably, there was no observed genotype switch in the selected SNP for SOD3 and GSR in all talc-treated cells. All cells, except for HOSEpiC cells, manifest the SNP genotype of

GPX1 (C/T). Intriguingly, talc treatment reversed this SNP genotype to the normal genotype (Table 2).

Talc Treatment Increased CA-125 Levels in Normal and EOC Cells

CA-125 ELISA assay was performed in protein isolated from cell media before and after talc treatment. CA-125 levels were significantly increased in a dose-dependent manner in all cells (Figure 4, $P < .05$). There was no detectable CA-125 protein in macrophages.

Talc Treatment Increased Cell Proliferation and Decreased Apoptosis

MTT cell proliferation assay was used to determine cell viability, and caspase-3 activity assay was utilized to determine apoptosis of all cell lines after 24 hours of talc treatment (Figure 5). Cell proliferation was significantly increased from the baseline in all talc-treated cells ($P < .05$), but to a greater degree in normal

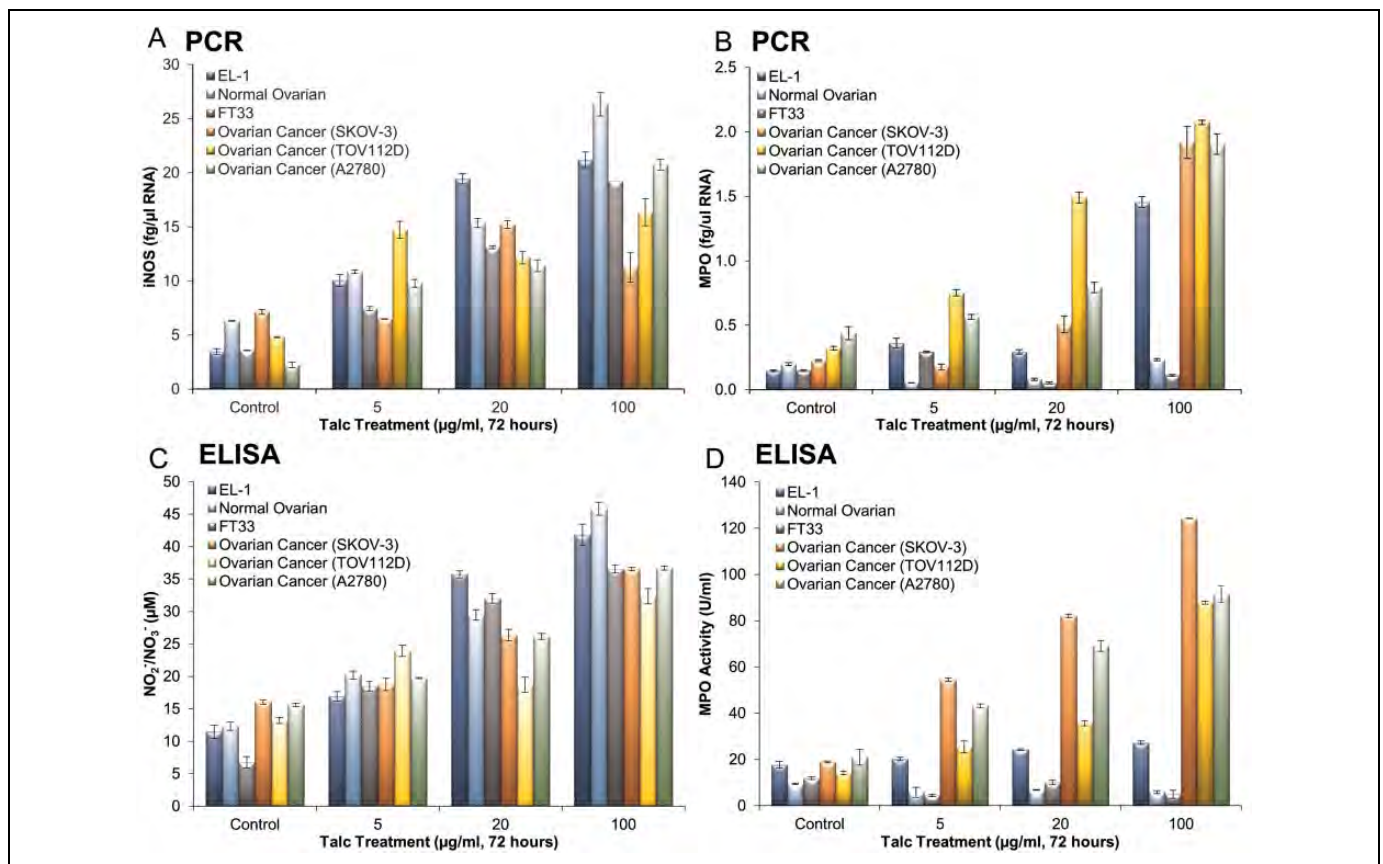


Figure 2. Increased expression and activity of key prooxidants, iNOS, NO₂⁻/NO₃⁻, and MPO. The mRNA (real time RT PCR) and protein/activity levels (ELISA) of iNOS (A and C) and MPO (B and D) were determined in macrophages (EL 1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV 3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. As expected, there was no detectable MPO in normal ovarian and fallopian tube cells, and thus, talc treatment did not have any effect. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in iNOS and MPO positive cells and in all doses as compared to controls. iNOS indicates inducible nitric oxide synthase; MPO, myeloperoxidase; mRNA, messenger RNA; RT PCR, reverse transcription polymerase chain reaction; ELISA, enzyme linked immunosorbent assay.

as compared to cancer cells. As anticipated, caspase-3 was significantly reduced in cancer as compared to normal cells. Talc treatment resulted in decreased caspase-3 activity in all cells as compared to controls (Figure 6, $P < .05$), indicating a decrease in apoptosis.

Discussion

The claim that regular use of talcum powder for hygiene purpose is associated with an increased risk of ovarian cancer is based on several reports confirming the presence of talc particles in the ovaries and other parts of the female reproductive tract as well as in lymphatic vessels and tissues of the pelvis.⁷⁻¹² The ability of talc particles to migrate through the genital tract to the distal fallopian tube and ovaries is well accepted.¹⁰ To date, the exact mechanism is not fully understood, though several studies have pointed toward the peristaltic pump feature of the uterus and fallopian tubes, which is known to enhance transport of sperm into the oviduct ipsilateral to the ovary bearing the dominant follicle.⁸⁻¹²

There are reports supporting the epidemiologic association of talc use and risk of ovarian cancer.^{11,12} Recent studies have shown that risks for EOC from genital talc use vary by histologic subtype, menopausal status at diagnosis, hormone therapy use, weight, and smoking. These observations suggest that estrogen and/or prolactin may play a role via macrophage activity and inflammatory response to talc. There has been debate as to the significance of the epidemiologic studies based on the fact that the reported epidemiologic risk of talc use and risk of ovarian cancer, although consistent, are relatively modest (30%-40%), and there is inconsistent increase in risk with duration of use. This observation is due, in part, to the challenges in quantifying exposure as well as the failure of epidemiological studies to obtain necessary information about the frequency and duration of usage.¹¹⁻¹³

In this study, we have shown beyond doubt that talc alters key redox and inflammatory markers, enhances cell proliferation, and inhibits apoptosis, which are hallmarks of ovarian cancer. More importantly, this effect is also manifested by talc in normal cells, including surface ovarian epithelium,

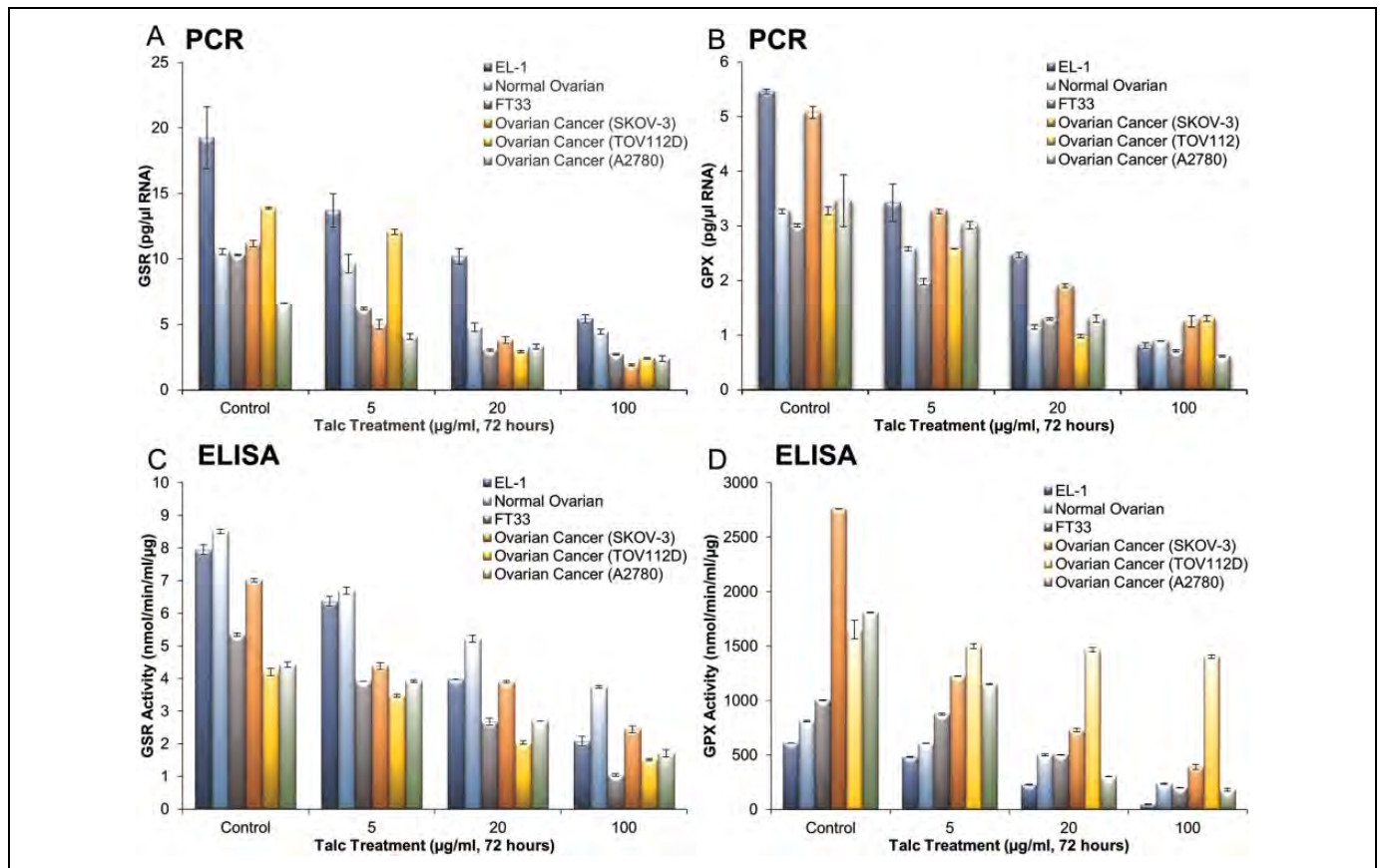


Figure 3. Decreased expression and activity of key antioxidant enzymes, GSR and GPX. The mRNA (real time RT PCR) and protein/activity levels (ELISA) of GSR (A and C) and GPX (B and D) were determined in macrophages (EL 1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV 3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells and in all doses as compared to controls. GSR indicates glutathione reductase; GPX, glutathione peroxidase; mRNA, messenger RNA; RT PCR, reverse transcription polymerase chain reaction; ELISA, enzyme linked immunosorbent assay.

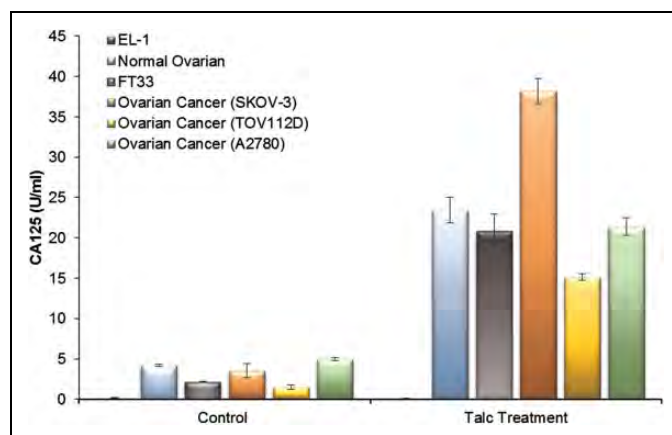
fallopian tube, and macrophages. Oxidative stress has been implicated in the pathogenesis of ovarian cancer, specifically by increased expression of several key prooxidant enzymes such as iNOS, MPO, and NAD(P)H oxidase in EOC tissues and cells as compared to normal cells indicating an enhanced redox state, as we have recently demonstrated (Figure 7).⁶ This redox state is further enhanced in chemoresistant EOC cells as evident by a further increase in iNOS and $\text{NO}_2^-/\text{NO}_3^-$ and a decrease in GSR levels, suggesting a shift toward a prooxidant state.⁶ Antioxidant enzymes, key regulators of cellular redox balance, are differentially expressed in various cancers, including ovarian.^{6,14} Specifically, GPX expression is reduced in prostate, bladder, kidney, and estrogen receptor negative breast cancer cell lines, though GPX is increased in other cancerous tissues from breast.¹⁴ Glutathione reductase levels, on the other hand, are elevated in lung cancer, although differentially expressed in breast and kidney cancer.^{5,15} Similarly, CAT was decreased in breast, bladder, and lung cancer while increased in brain cancer.¹⁶⁻¹⁸ Superoxide dismutase is expressed in lung, colorectal, gastric ovarian, and breast

cancer, while decreased activity and expression have been reported in colorectal carcinomas and pancreatic cancer cells.¹⁸⁻²¹ Collectively, this differential expression of antioxidants demonstrates the unique and complex redox microenvironment in cancer. Glutathione reductase is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to GSH. This enzyme is essential for the GSH redox cycle that maintains adequate levels of reduced cellular GSH. A high GSH to GSSG ratio is essential for protection against oxidative stress (Figure 5). Treatment with talc significantly reduced GSR in normal and cancer cells, altering the redox balance (Figure 3A and C). Likewise, GPX is an enzyme that detoxifies reactive electrophilic intermediates and thus plays an important role in protecting cells from cytotoxic and carcinogenic agents. Overexpression of GPX is triggered by exogenous chemical agents and reactive oxygen species and is thus thought to represent an adaptive response to stress.¹⁵ Indeed, treatment of normal and cancer cells with talc significantly reduced GPX, which compromised the overall cell response to stress (Figure 3B and D).

Table 2. SNP Characteristics (A) and SNP Genotyping of Key Redox Enzymes in Untreated and Talc Treated (100 µg/mL) Human Primary Ovarian Epithelial Cells (Normal Ovarian), Human Ovarian Surface Epithelial Cells (HOSEpiC), Fallopian Tube (FT33), and Ovarian Cancer (A2780, SKOV 3, TOV112D) Cell Lines (B).

	Gene (rs Number)				
	CAT (rs769217)	NOS ₂ (rs2297518)	GSR (rs8190955)	GPXI (rs3448)	SOD3 (rs2536512)
A					
MAF	0.123	0.173	0.191	0.176	0.476
SNP	C 262T	C2087T	G201T	C 1040T	A377T
Chromosome location	11p13	17q11.2	8p12	3q21.31	4p15.2
Amino acid switch	Isoleucine to Threonine	Serine to Leucine	Unknown	Unknown	Alanine to threonine
Effect on activity	Decrease	Increase	Unknown	Unknown	Decrease
B					
A2780: Control	C/C	C/C	G/G	C/T	A/A
A2780: Talc	C/C	C/C	G/G	C/C	A/A
SKOV 3: Control	C/C	C/C	G/G	C/T	A/A
SKOV 3: Talc	C/C	T/T	G/G	C/C	A/A
TOV112D: Control	C/C	C/C	G/G	C/T	A/A
TOV112D: Talc	C/T	C/C	G/G	C/C	A/A
HOSEpiC: Control	C/C	C/C	G/G	C/T	A/A
HOSEpiC: Talc	C/T	T/T	G/G	C/T	A/A
FT33: Control	C/C	C/C	G/G	C/T	A/A
FT33: Talc	C/T	T/T	G/G	C/C	A/A
Normal ovarian: Control	C/C	C/C	G/G	C/T	A/A
Normal ovarian: Talc	C/T	T/T	G/G	C/C	A/A

Abbreviation: SNP, single-nucleotide polymorphism.

**Figure 4.** Increased CA 125 levels in response to talc treatment. The level of ovarian cancer biomarker CA 125 was determined by ELISA before and after 72 hours of talc treatment (100 µg/mL) in macrophages (EL 1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV 3, TOV112D, and A2780) cells. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells as compared to controls. ELISA indicates enzyme linked immunosorbent assay.

We have previously reported that EOC cells manifest increased cell proliferations and decreased apoptosis.⁶ In this study, we have shown that talc enhances cell proliferation and induces an inhibition in apoptosis in EOC cells, but more importantly in normal cells, suggesting talc is a stimulus to the development of the oncogenic phenotype. We also previously

reported a cross talk between iNOS and MPO in ovarian cancer, which contributed to the lower apoptosis observed in ovarian cancer cells.^{6,22} Myeloperoxidase, an abundant hemoprotein, previously known to be present solely in neutrophils and monocytes, is a key oxidant enzyme that utilizes NO produced by iNOS as a 1-electron substrate generating NO⁺, a labile nitrosylating species.^{6,23,24} We were the first to report that MPO was expressed by EOC cells and tissues and that silencing MPO gene expression utilizing MPO-specific siRNA induced apoptosis in EOC cells through a mechanism that involved the S-nitrosylation of caspase-3 by MPO.²² Additionally, we have compelling evidence that MPO serves as a source of free iron under oxidative stress, where both NO⁺ and superoxide are elevated.⁶ Iron reacts with hydrogen peroxide (H₂O₂) and catalyzes the generation of highly reactive hydroxy radical (HO[•]), thereby increasing oxidative stress, which in turn increases free iron concentrations by the Fenton and Haber-Weiss reaction.^{6,24} We have previously highlighted the potential benefits of the combination of serum MPO and free iron as biomarkers for early detection and prognosis of ovarian cancer.²⁵ Collectively, we now have substantial evidence demonstrating that altered oxidative stress may play a role in maintaining the oncogenic phenotype of EOC cells. Treatment of normal or ovarian cancer cells with talc resulted in a significant increase in MPO and iNOS, supporting the role of talc in the enhancement of a prooxidant state that is a major cause in the development and maintenance of the oncogenic phenotype (Figure 2).

Furthermore, CA-125, which exists as a membrane-bound and secreted protein in EOC cells, has been established as a

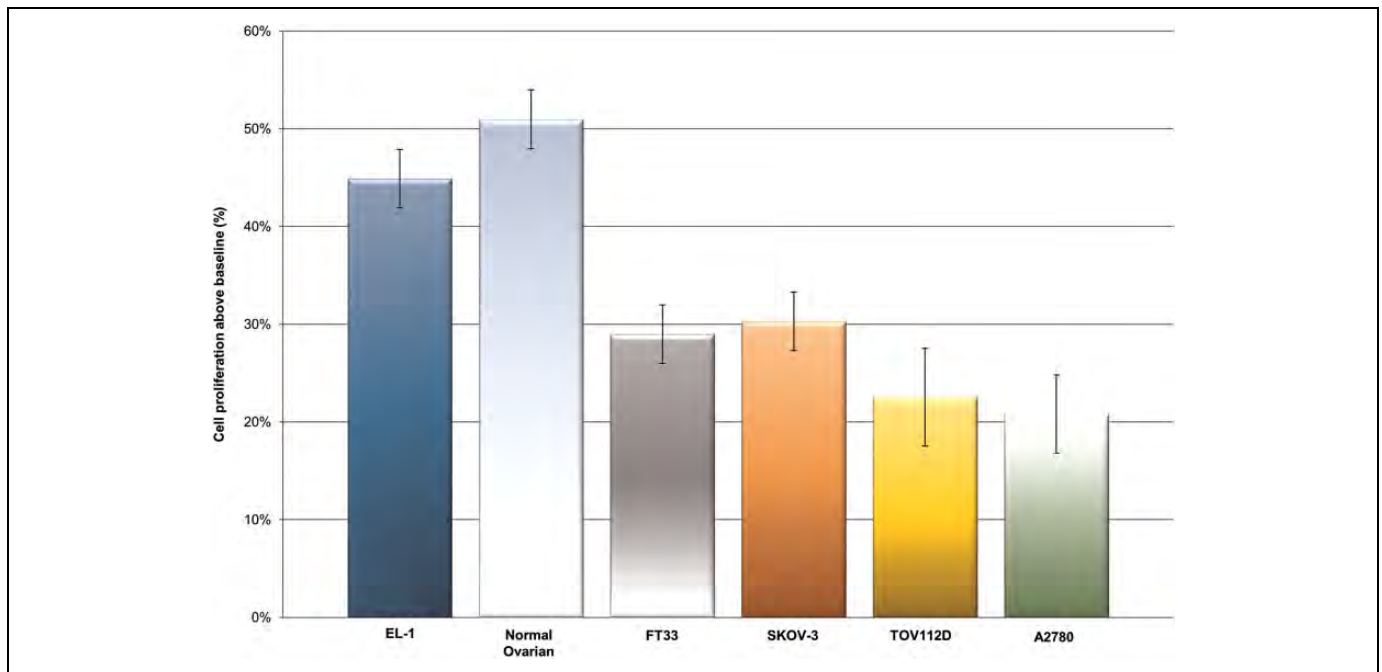


Figure 5. Increased cell proliferation in response to talc treatment. Cell proliferation was determined by MTT cell proliferation assay after 24 hours of talc treatment (100 µg/mL) in macrophages (EL 1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV 3, TOV112D, and A2780) cells. Experiments were performed in triplicate. Cell proliferation is depicted as the mean, with error bars representing standard deviation. All changes in response to talc treatment were significant ($P < .05$) in all cells as compared to controls.

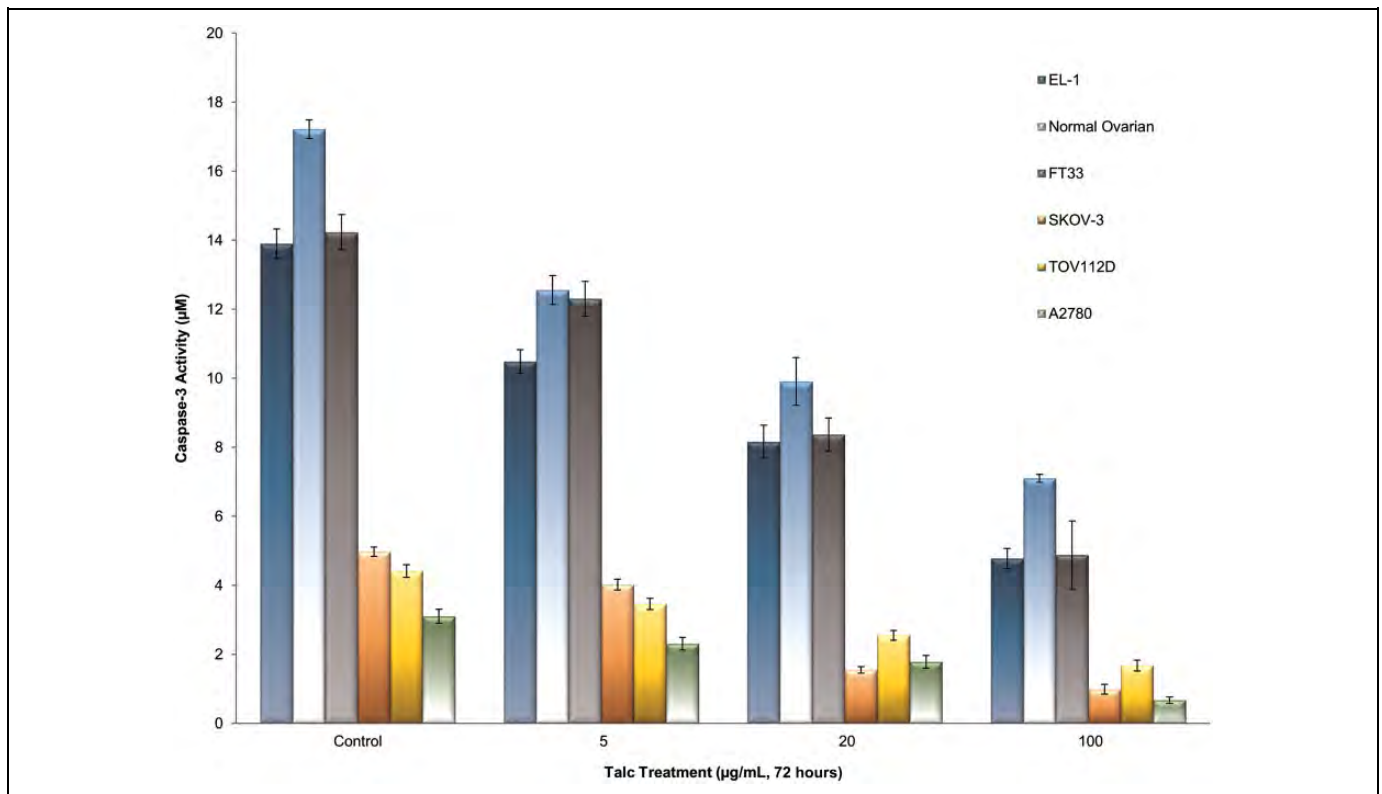


Figure 6. Decreased apoptosis in response to talc treatment. Caspase 3 activity was used to measure the degree of apoptosis in all cells. Caspase 3 activity assay was utilized to determine caspase 3 activity in macrophages (EL 1), human primary ovarian epithelial cells (normal ovarian), fallopian tube (FT33), and ovarian cancer (SKOV 3, TOV112D, and A2780) cell lines before and after treatment with various doses of talc over 72 hours. Experiments were performed in triplicate. Expression is depicted as the mean, with error bars representing standard error. All changes in response to talc treatment were significant ($P < .05$) in all cells and in all doses as compared to controls.

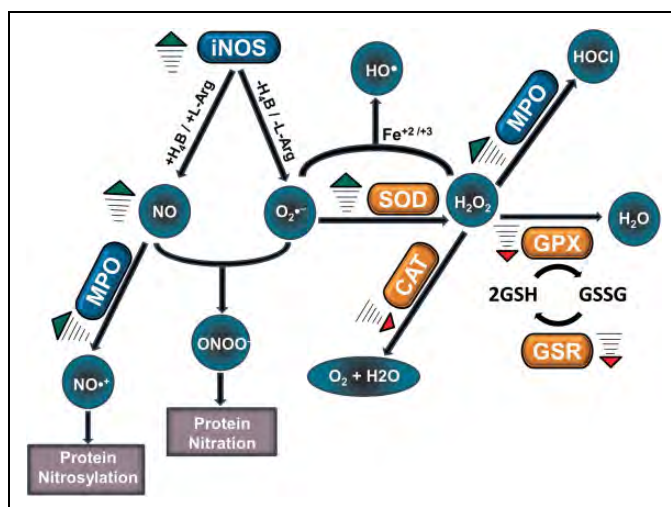


Figure 7. Epithelial ovarian cancer (EOC) cells have been reported to manifest a persistent prooxidant state as evident by the upregulation (green arrows) of key oxidants iNOS, NO, NO⁺, ONOO⁻, OH⁻, O₂⁺, and MPO (blue) and downregulation (red arrows) of key anti oxidants SOD, CAT, GPX, and GSR (orange). This redox state was also shown to be further enhanced in chemoresistant EOC cells. In this study, talcum powder altered the redox state, as indicated by the arrows, of both normal and EOC cells to create an enhanced prooxidant state. iNOS indicates inducible nitric oxide synthase; MPO, myeloperoxidase; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSR, glutathione reductase.

biomarker for disease progression and response to treatment.² CA-125 expression was significantly increased from nearly undetectable levels in controls to values approaching clinical significance (35 U/mL in postmenopausal women²⁶) in talc-treated cells (Figure 4, $P < .05$) without the physiologic effects on the tumor microenvironment one would expect to be present in the human body, thus highlighting the implications of the prooxidant states caused by talc alone.

To elucidate the mechanism by which talc alters the redox balance to favor a prooxidant state not only in ovarian cancer cells, but more importantly in normal cells, we have examined selected known gene mutations corresponding to SNPs known to be associated with altered enzymatic activity and increased cancer risk.^{6,27} Our results show that the *CAT* SNP (rs769217) resulting in decreased enzymatic activity was induced in all normal cell lines tested and in TOV112D EOC lines, but was not detected in A2780 or SKOV-3 cell lines (Table 2). Nevertheless, our results confirm a decrease in *CAT* expression and enzymatic activity in all talc-treated cells (Figure 1), indicating the existence of other *CAT* SNPs. The *SOD3* (rs2536512) and *GSR* (rs8190955) SNP genotypes were not detected in any cell line, yet *SOD3* and *GSR* activity and expression were decreased in all talc-treated cells, again suggesting the presence of other SNPs. Our results have also shown that all cells, except for HOSEpiC cells, manifest the SNP genotype of *GPX1* (C/T) before talc treatment. Intriguingly, talc treatment reversed this SNP genotype to the normal genotype (Table 2). Consistent with this finding, we have previously reported that acquisition

of chemoresistance by ovarian cancer cells is associated with a switch from the *GPX1* SNP genotype to the normal *GPX1* genotype.⁶ It is not understood why a *GPX1* SNP genotype predominates in untreated normal and ovarian cancer cells. Our results showed that talc treatment was associated with a genotype switch from common C/C genotype in *NOS2* in untreated cells to T/T, the SNP genotype, in talc-treated cells, except in A2780 and TOV112D (Table 2). Nevertheless, our results confirm an increase in iNOS expression and enzymatic activity in all talc-treated cells (Figure 2), again suggesting the existence of other *NOS2* SNPs. Collectively, these findings support the notion that talc treatment induced gene point mutations that happen to correspond to SNPs in locations with functional effects, thus altering overall redox balance for the initiation and development of ovarian cancer. Future studies examining such SNPs are important to fully elucidate a genotype switch mechanism induced by talc exposure.

In summary, this is the first study to clearly demonstrate that talc induces inflammation and alters the redox balance favoring a prooxidant state in normal and EOC cells. We have shown a dose-dependent significant increase in key prooxidants, iNOS, NO₂/NO₃, and MPO, and a concomitant decrease in key antioxidant enzymes, CAT, SOD, GPX, and GSR, in all talc-treated cells (both normal and ovarian cancer) compared to their controls. Additionally, there was a significant increase in CA-125 levels in all the talc-treated cells compared to their controls, except in macrophages. The mechanism by which talc alters the cellular redox and inflammatory balance involves the induction of specific mutations in key oxidant and antioxidant enzymes that correlate with alterations in their activities. The fact that these mutations happen to correspond to known SNPs of these enzymes indicate a genetic predisposition to developing ovarian cancer with genital talcum powder use.

Authors' Note

Ghassan M. Saed is also affiliated with Department of Gynecologic Oncology, Karmanos Cancer Institute, Detroit, MI, USA.

Acknowledgment

Special thanks to Imaan Singh for her technical contributions in acquiring the data and in development of graphics.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Saed has served as a paid consultant and expert witness in the talcum powder litigation.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Berek JS, Bertelsen K, du Bois A, et al. Epithelial ovarian cancer (advanced stage): consensus conference (1998) [in French]. *Gynecol Obstet Fertil*. 2000;28(7-8):576-583.

2. Jelovac D, Armstrong DK. Recent progress in the diagnosis and treatment of ovarian cancer. *CA Cancer J Clin*. 2011;61(3):183–203.
3. Prat J, Ribe A, Gallardo A. Hereditary ovarian cancer. *Hum Pathol*. 2005;36(8):861–870.
4. Ramus SJ, Vierkant RA, Johnatty SE, et al. Consortium analysis of 7 candidate SNPs for ovarian cancer. *Int J Cancer*. 2008;123(2):380–388.
5. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med*. 2010;49(11):1603–1616.
6. Fletcher NM, Belotte J, Saed MG, et al. Specific point mutations in key redox enzymes are associated with chemoresistance in epithelial ovarian cancer. *Free Radic Biol Med*. 2016;102:122–132.
7. Cramer DW, Welch WR, Scully RE, Wojciechowski CA. Ovarian cancer and talc: a case control study. *Cancer*. 1982;50:372–376.
8. Cramer DW, Liberman RF, Titus Ernstoff L, et al. Genital talc exposure and risk of ovarian cancer. *Int J Cancer*. 1999;81:351–356.
9. Ness RB, Grisso JA, Cotteau C, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. *Epidemiology*. 2000;11:111–117.
10. Henderson WJ, Joslin CA, Turnbull AC, Griffiths K. Talc and carcinoma of the ovary and cervix. *J Obstet Gynaecol Br Comm*. 1971;78:266–272.
11. Terry KL, Karageorgi S, Shvetsov YB, et al. Genital powder use and risk of ovarian cancer: a pooled analysis of 8,525 cases and 9,859 controls. *Cancer Prev Res (Phila)*. 2013;6(8):811–821.
12. Penninkilampi R, Eslick GD. Perineal talc use and ovarian cancer: a systematic review and meta analysis. *Epidemiology*. 2018;29(1):41–49.
13. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. *Cancer Biol Med*. 2017;14(1):9–32.
14. Brigelius Flohe R, Kipp A. Glutathione peroxidases in different stages of carcinogenesis. *Biochim Biophys Acta*. 2009;1790(11):1555–1568.
15. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic Biol Med*. 1990;8(6):583–599.
16. Popov B, Gadjeva V, Valkanov P, Popova S, Tolekova A. Lipid peroxidation, superoxide dismutase and catalase activities in brain tumor tissues. *Arch Physiol Biochem*. 2003;111(5):455–459.
17. Ray G, Batra S, Shukla NK, et al. Lipid peroxidation, free radical production and antioxidant status in breast cancer. *Breast Cancer Res Treat*. 2000;59(2):163–170.
18. Chung man Ho J, Zheng S, Comhair SA, Farver C, Erzurum SC. Differential expression of manganese superoxide dismutase and catalase in lung cancer. *Cancer Res*. 2001;61(23):8578–8585.
19. Radenkovic S, Milosevic Z, Konjevic G, et al. Lactate dehydrogenase, catalase, and superoxide dismutase in tumor tissue of breast cancer patients in respect to mammographic findings. *Cell Biochem Biophys*. 2013;66(2):287–295.
20. Hu Y, Rosen DG, Zhou Y, et al. Mitochondrial manganese superoxide dismutase expression in ovarian cancer: role in cell proliferation and response to oxidative stress. *J Biol Chem*. 2005;280(47):39485–39492.
21. Svensk AM, Soini Y, Paakko P, Hiravikoski P, Kinnula VL. Differential expression of superoxide dismutases in lung cancer. *Am J Clin Pathol*. 2004;122(3):395–404.
22. Saed GM, Ali Fehmi R, Jiang ZL, et al. Myeloperoxidase serves as a redox switch that regulates apoptosis in epithelial ovarian cancer. *Gynecol Oncol*. 2010;116(2):276–281.
23. Galijasevic S, Saed GM, Hazen SL, Abu Soud HM. Myeloperoxidase metabolizes thiocyanate in a reaction driven by nitric oxide. *Biochemistry*. 2006;45(4):1255–1262.
24. Galijasevic S, Maitra D, Lu T, Sliskovic I, Abdulhamid I, Abu Soud HM. Myeloperoxidase interaction with peroxynitrite: chloride deficiency and heme depletion. *Free Radic Biol Med*. 2009;47(4):431–439.
25. Fletcher NM, Jiang Z, Ali Fehmi R, et al. Myeloperoxidase and free iron levels: potential biomarkers for early detection and prognosis of ovarian cancer. *Cancer Biomark*. 2011;10(6):267–275.
26. Scholler N, Urban N. CA125 in ovarian cancer. *Biomark Med*. 2007;1(4):513–523.
27. Belotte J, Fletcher NM, Saed MG, et al. A single nucleotide polymorphism in catalase is strongly associated with ovarian cancer survival. *PLoS One*. 2015;10(8):e0135739.

Exhibit 97

Shawn Levy, Ph.D.

Page 1

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF NEW JERSEY

IN RE: JOHNSON & JOHNSON
TALCUM POWDER PRODUCTS
MARKETING, SALES PRACTICES,
AND PRODUCTS LIABILITY
LITIGATION

Case No. 16-2738
(FLW) (LHG)

THIS DOCUMENT RELATES TO
ALL CASES

MDL Docket No. 2738

Friday, January 11, 2019

- - - - -

The video deposition of SHAWN LEVY, Ph.D.,
taken pursuant to notice, was held at the
Embassy Suites Huntsville, 850 Monroe Street
S.W., Huntsville, Alabama, commencing at
approximately 9:04 a.m., on the above date,
before Lois Anne Robinson, Registered Diplomat
Reporter, Certified Realtime Reporter, and
Notary Public for the State of Alabama.

Shawn Levy, Ph.D.

Page 2	Page 4
<p>1 APPEARANCES</p> <p>2 COUNSEL FOR PLAINTIFFS' STEERING COMMITTEE:</p> <p>3 BEASLEY ALLEN, P C</p> <p>4 218 Commerce Street</p> <p>5 Montgomery, Alabama 36104</p> <p>6 BY: P LEIGH O'DELL, Esquire</p> <p>7 Leigh.odell@beasleyallen.com</p> <p>8 JENNIFER K EMMEL, ESQUIRE</p> <p>9 Jennifer.emmel@beasleyallen.com</p> <p>10 BURNS CHAREST, LLP</p> <p>11 900 Jackson Street, Suite 500</p> <p>12 Dallas, Texas 75202</p> <p>13 BY: MARTIN D BARRIE, J D , Ph D</p> <p>14 Mbarrie@burnscharest.com</p> <p>15 NAPOLI SHKOLNIK PLLC</p> <p>16 400 Broadhollow Road, Suite 305</p> <p>17 Melville, New York 11747</p> <p>18 BY: ALASTAIR J M FINDEIS, ESQUIRE</p> <p>19 Afindeis@napolilaw.com</p> <p>20 FOR THE DEFENDANT, JOHNSON & JOHNSON:</p> <p>21 WEIL, GOTSHAL & MANGES, LLP</p> <p>22 17 Hulfish Street, Suite 201</p> <p>23 Princeton, NJ 08542-3792</p> <p>24 BY: ALLISON M BROWN, ESQUIRE</p> <p>Allison.brown@weil.com</p> <p>WEIL, GOTSHAL & MANGES, LLP</p> <p>767 Fifth Avenue</p> <p>New York, New York 10153-0119</p> <p>BY: ALEXIS KELLERT, ESQUIRE</p> <p>Alexis.kellert@weil.com</p> <p>SKADDEN, ARPS, SLATE, MEAGHER & FLOM, LLP</p> <p>4 Times Square</p> <p>New York, New York 10036</p> <p>BY: Benjamin Halperin, Esquire</p> <p>Benjamin.halperin@skadden.com</p>	<p>1 INDEX</p> <p>2 EXAMINATION PAGE</p> <p>3</p> <p>4 By Ms. Brown 7</p> <p>5 By Mr. Ferguson 307</p> <p>6 By Ms. O'Dell 357</p> <p>7 By Ms. Brown 372</p> <p>8 By Ms. O'Dell 389</p> <p>9</p> <p>10 * * * * *</p> <p>11</p> <p>12 EXHIBITS</p> <p>13 Deposition Exhibit Number 1 14</p> <p>14 Notice of Deposition</p> <p>15 Deposition Exhibit Number 2 33</p> <p>16 Levy expert report</p> <p>17 Deposition Exhibit Number 3 16</p> <p>18 Levy invoices of 5/2/18 and 1/8/19</p> <p>19 Deposition Exhibit Number 4 19</p> <p>20 Government of Canada document regarding draft screening</p> <p>21 assessment of talc</p> <p>22 Deposition Exhibit Number 5 21</p> <p>23 Government of Canada document regarding potential risk of</p> <p>24 lung effects and ovarian cancer from talc</p>
Page 3	Page 5
<p>1 APPEARANCES - (continued)</p> <p>2 FOR THE DEFENDANT, IMERYS TALC AMERICA:</p> <p>3 GORDON & REES SCULLY MANSUKHANI, LLP</p> <p>4 816 Congress Avenue, Suite 1510</p> <p>5 Austin, Texas 78701</p> <p>6 BY: KENNETH J. FERGUSON, ESQUIRE</p> <p>7 Kferguson@gordonrees.com</p> <p>8 COUNSEL FOR PTI:</p> <p>9 TUCKER ELLIS, LLP</p> <p>10 233 S. Wacker Drive, Suite 6950</p> <p>11 Chicago, Illinois 60606-9997</p> <p>12 BY: JAMES W. MIZGALA, ESQUIRE</p> <p>13 James.mizgala@tuckerellis.com</p> <p>14 COUNSEL FOR PERSONAL CARE PRODUCTS COUNCIL:</p> <p>15 SEYFARTH SHAW LLP</p> <p>16 975 F Street N.W.</p> <p>17 Washington, D.C. 20004-1454</p> <p>18 BY: RENÉE B. APPEL, ESQUIRE</p> <p>19 Rappel@seyfarth.com</p> <p>20 VIDEOGRAPHER:</p> <p>21 JULIE ROBINSON</p> <p>22 LOIS ANNE ROBINSON, RPR, RDR, CRR</p> <p>23 COURT REPORTER</p> <p>24</p>	<p>1 INDEX - (Continued)</p> <p>2 Deposition Exhibit Number 6 23</p> <p>3 Draft manuscript regarding systematic review and</p> <p>4 meta-analysis of the association between perineal use of talc</p> <p>5 and risk of ovarian cancer</p> <p>6 Deposition Exhibit Number 7 30</p> <p>7 Hamilton article</p> <p>8 Deposition Exhibit Number 8 49</p> <p>9 Judith Zelikoff expert report</p> <p>10 Deposition Exhibit Number 9 59</p> <p>11 Mayo Clinic website article entitled "Cancer"</p> <p>12 Deposition Exhibit Number 10 72</p> <p>13 Wikipedia page</p> <p>14 Deposition Exhibit Number 11 75</p> <p>15 Coussens and Werb article</p> <p>16 Deposition Exhibit Number 12 82</p> <p>17 Preprint manuscript of "Molecular Basis Supporting the</p> <p>18 Association of Talcum Powder Use With Increased Risk of</p> <p>19 Ovarian Cancer"</p> <p>20 Deposition Exhibit Number 13 82</p> <p>21 December 26 Email to Dr. Saed</p> <p>22 Deposition Exhibit Number 14 142</p> <p>23 "Evaluating Biological Plausibility in Supporting Evidence</p> <p>24 For Action Through Systematic Reviews in Public Health"</p>

Shawn Levy, Ph.D.

Page 6	Page 8
<p>1 INDEX - (continued)</p> <p>2 Deposition Exhibit Number 15 190</p> <p>3 NTP study</p> <p>4 Deposition Exhibit Number 16 192</p> <p>5 2014 Citizens Petition to FDA</p> <p>6 Deposition Exhibit Number 17 208</p> <p>7 Buz/Zard study</p> <p>8 Deposition Exhibit Number 18 218</p> <p>9 "Perineal Talc Use and Ovarian Cancer," by Ross Penninkilampi</p> <p>10 Deposition Exhibit Number 19 249</p> <p>11 Heller article</p> <p>12 Deposition Exhibit Number 20 270</p> <p>13 Merritt paper - "Talcum Powder Chronic Pelvic Inflammation</p> <p>14 and NSAIDs in Relation to the Risk of Epithelial Ovarian</p> <p>15 Cancer"</p> <p>16 Deposition Exhibit Number 21 326</p> <p>17 Nunes article</p> <p>18 Deposition Exhibit Number 22 367</p> <p>19 Park article</p> <p>20</p> <p>21</p> <p>22</p> <p>23</p> <p>24</p>	<p>1 A Good morning.</p> <p>2 Q My name is Alli Brown. I represent</p> <p>3 Johnson & Johnson, and I'll start with some</p> <p>4 questions for you here today.</p> <p>5 Dr. Levy, have you ever been deposed</p> <p>6 before?</p> <p>7 A Yes.</p> <p>8 Q And tell me, how many times?</p> <p>9 A In a setting like this, once.</p> <p>10 Q Okay. What was the nature of that</p> <p>11 deposition?</p> <p>12 A It was a patent litigation case.</p> <p>13 Q Were you serving as an expert witness</p> <p>14 in that case?</p> <p>15 A I was.</p> <p>16 Q Were you hired by the plaintiffs or the</p> <p>17 defendants?</p> <p>18 A The plaintiffs.</p> <p>19 Q And, just generally, what were the</p> <p>20 issues in that case?</p> <p>21 A It was entirely focused on evaluation</p> <p>22 of prior art in the genomic space.</p> <p>23 Q And any time --</p> <p>24 And do you remember the name of that</p>
Page 7	Page 9
<p>1 VIDEOGRAPHER:</p> <p>2 We are now on the record. My name is</p> <p>3 Julie Robinson. I'm a videographer representing</p> <p>4 Golkow Litigation Services.</p> <p>5 Today's date is January 11th, 2019, and</p> <p>6 the time is 9:04 a.m.</p> <p>7 This video deposition is being held in</p> <p>8 Huntsville, Alabama, in the matter of</p> <p>9 Johnson & Johnson Talcum Powder Product Marketing,</p> <p>10 Sales Practices, and Products Liability</p> <p>11 Litigation, MDL Docket Number 2738.</p> <p>12 The deponent is Dr. Shawn Levy.</p> <p>13 Counsel will be noted on the</p> <p>14 stenographic record.</p> <p>15 The court reporter is Lois Robinson,</p> <p>16 who will now swear in the witness.</p> <p>17 SHAWN LEVY, Ph.D.,</p> <p>18 the witness, after having first been</p> <p>19 duly sworn to tell the truth, the whole truth,</p> <p>20 and nothing but the truth, was examined and</p> <p>21 testified as follows:</p> <p>22 EXAMINATION</p> <p>23 BY MS. BROWN:</p> <p>24 Q Good morning, Dr. Levy.</p>	<p>1 case, by the way?</p> <p>2 A I don't. It was, gosh, twelve years</p> <p>3 ago or so.</p> <p>4 Q I see.</p> <p>5 Did that case go to trial?</p> <p>6 A Not that I'm aware of.</p> <p>7 Q Have you ever testified at trial?</p> <p>8 A I have not.</p> <p>9 Q Okay. And other than that one patent</p> <p>10 case you just described for us, were there other</p> <p>11 depositions that you've given?</p> <p>12 A No.</p> <p>13 Q And I think, when you started to answer</p> <p>14 the question in the beginning, you said "in a</p> <p>15 setting like this." Is there another time, in</p> <p>16 your mind, where you've given testimony under</p> <p>17 oath?</p> <p>18 A No, not under oath. That's why I</p> <p>19 was --</p> <p>20 So I've had a number of meetings, all</p> <p>21 limited to the patent space of mainly prior art</p> <p>22 discussions, where there's been representatives</p> <p>23 from both sides where we were having a</p> <p>24 discussion. But it wasn't a formal deposition</p>

3 (Pages 6 to 9)

Shawn Levy, Ph.D.

Page 10	Page 12
<p>1 with a court reporter, under oath, et cetera.</p> <p>2 Q Understood.</p> <p>3 So this would then be the second time</p> <p>4 you've been deposed in a setting like this.</p> <p>5 A Correct.</p> <p>6 Q Is that fair?</p> <p>7 Okay. So a few ground rules that you</p> <p>8 may already be familiar with from your prior</p> <p>9 experience. First, we'll try not to speak over</p> <p>10 each other. Is that fair?</p> <p>11 A That's fair.</p> <p>12 Q That way, our court reporter can get</p> <p>13 down all my questions and all your answers.</p> <p>14 Okay?</p> <p>15 A (Nods affirmatively.)</p> <p>16 Q If you don't understand a question of</p> <p>17 mine, will you let me know?</p> <p>18 A I will.</p> <p>19 Q Okay. Try to verbalize your answers,</p> <p>20 too, so our court reporter can take them down.</p> <p>21 Okay?</p> <p>22 A Understood.</p> <p>23 Q Okay. If you need a break, let me</p> <p>24 know, and we'll be happy to accommodate you.</p>	<p>1 with.</p> <p>2 Q Okay. In front of you is the</p> <p>3 plaintiffs' lawyer's laptop. Is that right?</p> <p>4 A That's right.</p> <p>5 Q Okay. And what is contained on the</p> <p>6 plaintiffs' lawyer's laptop?</p> <p>7 MS. O'DELL:</p> <p>8 I think I'd probably be better to speak</p> <p>9 to it.</p> <p>10 MS. BROWN:</p> <p>11 No, no. Let's get it from the witness,</p> <p>12 and then if you want to make a statement for the</p> <p>13 record, of course.</p> <p>14 Q Let's -- let's get your understanding</p> <p>15 of what's on this laptop in front of you.</p> <p>16 A Other than what's on the USB drive that</p> <p>17 I've been using, I -- I don't have any knowledge</p> <p>18 of what's on it.</p> <p>19 Q Okay. Do you know what's on the USB</p> <p>20 drive?</p> <p>21 A I do.</p> <p>22 Q What's that?</p> <p>23 A It's a collection of literature cited</p> <p>24 in reliance literature list that -- from</p>
Page 11	Page 13
<p>1 Do you understand you're under oath</p> <p>2 here today, same as if you were in a court of</p> <p>3 law?</p> <p>4 A I do.</p> <p>5 Q Okay. I am --</p> <p>6 And, before we get started, Doctor, I</p> <p>7 see you have a couple of items in front of you,</p> <p>8 and I want to identify what we have for the</p> <p>9 record.</p> <p>10 To your right is an iPad that is</p> <p>11 showing the realtime of my questions and your</p> <p>12 answers. Will you be using that to assist you in</p> <p>13 your testimony here today?</p> <p>14 A Yes.</p> <p>15 Q Okay. In front of you you have a</p> <p>16 laptop computer.</p> <p>17 A (Nods affirmatively.)</p> <p>18 Q Will you be using that to assist you in</p> <p>19 your testimony?</p> <p>20 A Yes.</p> <p>21 Q And tell me, is this your laptop?</p> <p>22 A It is not.</p> <p>23 Q Okay. Whose laptop is it?</p> <p>24 A The -- the attorneys I've been working</p>	<p>1 my -- from my report.</p> <p>2 Q Did you put together the items that are</p> <p>3 contained on the USB drive that you have in front</p> <p>4 of you?</p> <p>5 MS. O'DELL:</p> <p>6 Object to the form.</p> <p>7 A Yes.</p> <p>8 MS. BROWN:</p> <p>9 Q Is that your USB drive?</p> <p>10 A No. I put together the list.</p> <p>11 As far as who moved the files and</p> <p>12 organized the files on the USB, that, I don't</p> <p>13 know.</p> <p>14 Q Okay. Are all of the files on that USB</p> <p>15 drive documents that you considered in connection</p> <p>16 with your opinion in this case?</p> <p>17 A They are.</p> <p>18 Q Any other materials in front of you</p> <p>19 that you'll be using to assist you in your</p> <p>20 testimony here today?</p> <p>21 A There's a -- I have a hard copy of my</p> <p>22 report.</p> <p>23 Q Did you prepare that hard copy binder?</p> <p>24 A No.</p>

4 (Pages 10 to 13)

Shawn Levy, Ph.D.

Page 14	Page 16
<p>1 Q Who -- who did?</p> <p>2 A My -- the -- the attorneys I've been</p> <p>3 working with. So I -- they -- they provided the</p> <p>4 printout and the nice binder that it's in.</p> <p>5 Q Okay. Did you, Doctor, make any notes</p> <p>6 on the report that you have in front of you?</p> <p>7 A No.</p> <p>8 Q Okay. I'm gonna hand you what we have</p> <p>9 marked as Exhibit 1 to your deposition, which is</p> <p>10 a notice of your deposition.</p> <p>11 (DEPOSITION EXHIBIT NUMBER 1</p> <p>12 WAS MARKED FOR IDENTIFICATION.)</p> <p>13 MS. BROWN:</p> <p>14 Q And I'll ask, is this something that</p> <p>15 you have ever seen before?</p> <p>16 A Yes.</p> <p>17 Q When did you see it?</p> <p>18 A I'd have to review my email, but it was</p> <p>19 some -- sometime ago, some weeks ago.</p> <p>20 Q Okay. Have you brought any --</p> <p>21 And you understand that this Notice of</p> <p>22 Deposition that we've marked as Exhibit 1</p> <p>23 requests that you bring certain documents with</p> <p>24 you here today?</p>	<p>1 Thank you.</p> <p>2 -- by marking these, and I'll ask you</p> <p>3 some questions about what we have.</p> <p>4 (DEPOSITION EXHIBIT NUMBER 3</p> <p>5 WAS MARKED FOR IDENTIFICATION.)</p> <p>6 MS. BROWN:</p> <p>7 Q I'll mark as Exhibit 3 to your</p> <p>8 deposition two invoices counsel for plaintiffs</p> <p>9 just handed me, one dated May 2nd, 2018, and the</p> <p>10 other dated January 8th, 2019. And we only have</p> <p>11 one copy, so let me hand it to you and ask you,</p> <p>12 are these invoices that you created, Doctor?</p> <p>13 A They are.</p> <p>14 Q Okay. And I want to take that back for</p> <p>15 one second.</p> <p>16 Looks like the first entry on your</p> <p>17 invoice is dated May 16th, 2017. Does that sound</p> <p>18 right to you?</p> <p>19 A That sounds right.</p> <p>20 Q When were you first approached about an</p> <p>21 involvement in this case?</p> <p>22 A Earlier in 2017.</p> <p>23 Q Okay. And who approached you?</p> <p>24 A Leigh and Jennifer. I'd have to verify</p>
Page 15	Page 17
<p>1 A Yes.</p> <p>2 Q Okay.</p> <p>3 MS. O'DELL:</p> <p>4 Let me just insert for the record,</p> <p>5 we've objected to certain requests contained in</p> <p>6 the notice, and objections have been served, and</p> <p>7 materials have been brought to this deposition</p> <p>8 consistent with those objections.</p> <p>9 MS. BROWN:</p> <p>10 And we are in receipt of your</p> <p>11 objections.</p> <p>12 Q And your counsel for the plaintiffs</p> <p>13 represented that some materials have been brought</p> <p>14 to the deposition. Do you have any materials</p> <p>15 with you responsive to this notice?</p> <p>16 A Well --</p> <p>17 MS. O'DELL:</p> <p>18 I'll provide to you invoices that are</p> <p>19 responsive to the Notice, and there are materials</p> <p>20 that Dr. Levy has seen since his report was</p> <p>21 served, and -- and those are copies.</p> <p>22 MS. BROWN:</p> <p>23 Thank you, counsel.</p> <p>24 Q So, Doctor, let's start --</p>	<p>1 in my email whom I may have heard from first.</p> <p>2 Q Okay. And Leigh and Jennifer are</p> <p>3 counsel for plaintiffs in this litigation; is</p> <p>4 that right?</p> <p>5 A That's right.</p> <p>6 Q And did they -- had you known them</p> <p>7 prior to receiving contact early in 2017 --</p> <p>8 A No.</p> <p>9 Q -- from plaintiffs' lawyers?</p> <p>10 A I -- I did not know them.</p> <p>11 Q Did they call you at your place of</p> <p>12 business?</p> <p>13 A I believe the first contact was email.</p> <p>14 But, ultimately, yes.</p> <p>15 Q Okay. And was there any connection,</p> <p>16 meaning did someone refer the plaintiffs' lawyers</p> <p>17 to you, or do you know?</p> <p>18 A I don't know.</p> <p>19 Q Do you have any idea how the</p> <p>20 plaintiffs' lawyers found you?</p> <p>21 A I do not.</p> <p>22 Q Okay. It looks like, Doctor, that</p> <p>23 these two invoices have a total of 33 hours.</p> <p>24 Does that sound right to you?</p>

5 (Pages 14 to 17)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 18</p> <p>1 A It does.</p> <p>2 Q Looks like something's blacked out on</p> <p>3 the second page of the invoices. Do you know</p> <p>4 what that is?</p> <p>5 MS. O'DELL:</p> <p>6 I'll just say that redactions were made</p> <p>7 by counsel. They referenced the subject matter</p> <p>8 of conversations between Dr. Levy and counsel,</p> <p>9 and those have been redacted because of work</p> <p>10 product privilege.</p> <p>11 MS. BROWN:</p> <p>12 Okay.</p> <p>13 Q Is it fair, Doctor, that you've spent a</p> <p>14 total of 33 hours forming your opinions in this</p> <p>15 case?</p> <p>16 A That's fair.</p> <p>17 Q Okay. Do you have any additional</p> <p>18 invoices that you plan to submit to the lawyers</p> <p>19 for the plaintiffs?</p> <p>20 A Yes.</p> <p>21 Q Okay. And can you ballpark for me how</p> <p>22 much additional time you've spent since the last</p> <p>23 entry here, which appears to be December 12th,</p> <p>24 2018?</p>	<p style="text-align: right;">Page 20</p> <p>1 Your report in this case was served in</p> <p>2 November of 2018; correct?</p> <p>3 A Correct.</p> <p>4 Q Fair to say, then, that Exhibit 4,</p> <p>5 which you saw for the first time in December of</p> <p>6 2018, did not inform the opinions contained in</p> <p>7 your report?</p> <p>8 A That's correct.</p> <p>9 Q Okay. Did the -- does Exhibit 4</p> <p>10 contain any information regarding chronic</p> <p>11 inflammation as the proposed mechanism of ovarian</p> <p>12 cancer induced by talc?</p> <p>13 A I don't believe it does. I'd have to</p> <p>14 review -- take a look at it to be sure.</p> <p>15 MS. O'DELL:</p> <p>16 And if you need to look at it, I'm sure</p> <p>17 counsel will hand it to you.</p> <p>18 MS. BROWN:</p> <p>19 Q I'm handing you, Doctor --</p> <p>20 MS. O'DELL:</p> <p>21 Excuse me. If you need to look at it</p> <p>22 to answer that question, you may.</p> <p>23 A To be sure I'm accurate in my answer,</p> <p>24 I'd like to take a look at that.</p>
<p style="text-align: right;">Page 19</p> <p>1 A There's probably another -- not</p> <p>2 including this morning -- roughly 15 hours.</p> <p>3 Okay. I'll hand you, Doctor, what we</p> <p>4 have marked as Exhibit 4 to your deposition.</p> <p>5 This is another document counsel for the</p> <p>6 plaintiffs just handed me.</p> <p>7 (DEPOSITION EXHIBIT NUMBER 4</p> <p>8 WAS MARKED FOR IDENTIFICATION.)</p> <p>9 MS. BROWN:</p> <p>10 Q Would you identify that for the record,</p> <p>11 please.</p> <p>12 A This is a printed copy from a website</p> <p>13 from the government of Canada discussing their</p> <p>14 draft screening assessment of talc.</p> <p>15 Q Okay. Is that something you've seen</p> <p>16 before today?</p> <p>17 A Yes.</p> <p>18 Q When did you see it first?</p> <p>19 A Sometime in December.</p> <p>20 Q Did the lawyers for plaintiffs give it</p> <p>21 to you?</p> <p>22 A They did.</p> <p>23 Q Okay. Your report in this case --</p> <p>24 Can I have that back?</p>	<p style="text-align: right;">Page 21</p> <p>1 MS. BROWN:</p> <p>2 Q Sure. Sitting here --</p> <p>3 Hold on.</p> <p>4 Sitting here today, you're not aware if</p> <p>5 Exhibit 4 contains any information regarding the</p> <p>6 proposed mechanism of chronic inflammation as a</p> <p>7 cause for ovarian cancer?</p> <p>8 MS. O'DELL:</p> <p>9 Object to the question.</p> <p>10 If you need to see the document,</p> <p>11 Doctor, you may ask for it.</p> <p>12 A Yeah. I'm not -- I'm not able to</p> <p>13 answer it accurately without seeing the document.</p> <p>14 (DEPOSITION EXHIBIT NUMBER 5</p> <p>15 WAS MARKED FOR IDENTIFICATION.)</p> <p>16 MS. BROWN:</p> <p>17 Q Okay. Handing you what we've marked as</p> <p>18 Exhibit 5, would you tell me what that is,</p> <p>19 Doctor?</p> <p>20 A This is another document from the</p> <p>21 government -- government of Canada discussing the</p> <p>22 potential risk of lung effects and ovarian cancer</p> <p>23 from talc.</p> <p>24 Q Is Exhibit 5 a final document, do you</p>

6 (Pages 18 to 21)

Shawn Levy, Ph.D.

Page 22	Page 24
<p>1 know?</p> <p>2 MS. O'DELL:</p> <p>3 Object to the form.</p> <p>4 A Yeah. That -- I don't -- I don't have</p> <p>5 the information available to answer that</p> <p>6 accurately.</p> <p>7 MS. BROWN:</p> <p>8 Q Have you seen Exhibit 5 prior to this</p> <p>9 morning?</p> <p>10 A I have.</p> <p>11 Q When did you first see Exhibit 5?</p> <p>12 A Similar in time to the earlier report</p> <p>13 or this -- yes. Similar in time to the</p> <p>14 earlier -- to the same document from Exhibit 4.</p> <p>15 Q To the best of your recollection,</p> <p>16 Doctor, you first saw Exhibit 5 after completing</p> <p>17 your report in this matter; is that right?</p> <p>18 A That is right.</p> <p>19 Q Fair to say, then, that Exhibit 5 did</p> <p>20 not inform the opinions contained in your MDL</p> <p>21 report?</p> <p>22 A That's correct.</p> <p>23 Q Handing you, Doctor, what we've marked</p> <p>24 as Exhibit 6 to your deposition, another document</p>	<p>1 Q Does Exhibit 6 contain any information</p> <p>2 regarding the proposed mechanism of chronic</p> <p>3 inflammation?</p> <p>4 A It does in reference, I believe. I'm</p> <p>5 reminding myself if -- if it shared the same</p> <p>6 materials that I had referenced in my report.</p> <p>7 So, yes, it does.</p> <p>8 Q Are you looking at a particular page,</p> <p>9 Doctor?</p> <p>10 A I am.</p> <p>11 Q And would you identify that for the</p> <p>12 record.</p> <p>13 A I'm looking at page 23, beginning at</p> <p>14 line 220.</p> <p>15 Q And what information does Exhibit 6 at</p> <p>16 page 23 contain regarding chronic inflammation?</p> <p>17 A It discusses inflammation of the</p> <p>18 epithelial ovarian surfaces in animal models and</p> <p>19 provides two different references.</p> <p>20 Q And were those references information</p> <p>21 you considered in forming your opinions in this</p> <p>22 case?</p> <p>23 A Let me make sure of that.</p> <p>24 Yes.</p>
Page 23	Page 25
<p>1 counsel provided, counsel for plaintiffs provided</p> <p>2 in response to your deposition notice.</p> <p>3 (DEPOSITION EXHIBIT NUMBER 6</p> <p>4 WAS MARKED FOR IDENTIFICATION.)</p> <p>5 MS. BROWN:</p> <p>6 Q Would you identify for the record</p> <p>7 Exhibit 6?</p> <p>8 A So this is a draft manuscript or</p> <p>9 preprint manuscript that's been submitted for</p> <p>10 peer review discussing the systematic review and</p> <p>11 meta-analysis of the association between perineal</p> <p>12 use of talc and risk of ovarian cancer.</p> <p>13 Q Had you seen Exhibit 6 prior to this</p> <p>14 morning?</p> <p>15 A Yes.</p> <p>16 Q When did you first see Exhibit 6?</p> <p>17 A It was in December as well.</p> <p>18 Q Exhibit 6 did not inform your opinions</p> <p>19 in this matter. Fair?</p> <p>20 A They did not inform the content of the</p> <p>21 report.</p> <p>22 Q Have you reviewed and analyzed Exhibit</p> <p>23 6 since December?</p> <p>24 A I have.</p>	<p>1 Q And would you state what they are for</p> <p>2 the record, please?</p> <p>3 A One reference is T.C. Hamilton, et al.,</p> <p>4 The British Journal of Experimental Pathology,</p> <p>5 from 1984.</p> <p>6 And the other reference is "The</p> <p>7 Pathology of Ovarian" -- "The Pathology of</p> <p>8 Ovarian Cancer Precursors," which is a review of</p> <p>9 R.E. Scully in the Journal of Cellular</p> <p>10 Biochemistry, and that is a supplement from 1995.</p> <p>11 The latter is not referenced in my report.</p> <p>12 Q Have you reviewed the Scully paper in</p> <p>13 connection with your opinions in this matter?</p> <p>14 A Not specifically, no.</p> <p>15 Q You have, however, reviewed the</p> <p>16 Hamilton paper?</p> <p>17 A Yes.</p> <p>18 Q You would agree that the Hamilton paper</p> <p>19 does not show inflammation leading to neoplastic</p> <p>20 changes in animals?</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A I'd have to see the manu- -- or the</p> <p>24 manuscript to answer your specific question</p>

7 (Pages 22 to 25)

Shawn Levy, Ph.D.

Page 26	Page 28
<p>1 regarding neoplasm.</p> <p>2 MS. BROWN:</p> <p>3 Q Does the Hamilton paper support your</p> <p>4 view that chronic inflammation is a plausible</p> <p>5 mechanism for talc-induced ovarian cancer?</p> <p>6 A It supports my opinion that</p> <p>7 inflammation is a component in the progression to</p> <p>8 ovarian cancer.</p> <p>9 Q Is it your testimony that the Hamilton</p> <p>10 paper supports your opinion that chronic</p> <p>11 inflammation leads to neoplastic changes?</p> <p>12 A No, not necessarily.</p> <p>13 Q Okay. Tell me how it is that the</p> <p>14 Hamilton paper supports your opinion that chronic</p> <p>15 inflammation can cause ovarian cancer.</p> <p>16 A Well, the -- so my opinion regarding --</p> <p>17 that the role of inflammation in ovarian cancer</p> <p>18 is not based on a single study, particularly one</p> <p>19 that is now approaching or is now over 30 years</p> <p>20 old.</p> <p>21 Q Okay. Does --</p> <p>22 A So it's a -- I reviewed the -- that</p> <p>23 paper as well as a large number or the totality</p> <p>24 of the available evidence stretching across many</p>	<p>1 MS. O'DELL:</p> <p>2 -- paper in order to answer the</p> <p>3 question --</p> <p>4 MS. BROWN:</p> <p>5 Counsel --</p> <p>6 MS. O'DELL:</p> <p>7 -- you may do that.</p> <p>8 MS. BROWN:</p> <p>9 Counsel, he is absolutely entitled to</p> <p>10 get the paper. We're going to do that.</p> <p>11 Q Sitting here today, do you recall --</p> <p>12 MS. O'DELL:</p> <p>13 But he is not --</p> <p>14 MS. BROWN:</p> <p>15 It's a fair question.</p> <p>16 MS. O'DELL:</p> <p>17 Is it not a fair question.</p> <p>18 MS. BROWN:</p> <p>19 I'm not gonna --</p> <p>20 MS. O'DELL:</p> <p>21 He's asking --</p> <p>22 MS. BROWN:</p> <p>23 -- do this with you.</p> <p>24 MS. O'DELL:</p>
Page 27	Page 29
<p>1 years to develop the opinion that's represented</p> <p>2 in my report.</p> <p>3 Q Sure.</p> <p>4 A And to that opinion is -- no one study</p> <p>5 or one singular piece of information is the basis</p> <p>6 of that opinion.</p> <p>7 Q Okay. But, you know, having reviewed</p> <p>8 Hamilton, that what Hamilton shows is that the</p> <p>9 inflammation they saw in the animals was not</p> <p>10 associated with neoplastic changes. Right?</p> <p>11 MS. O'DELL:</p> <p>12 Excuse me.</p> <p>13 Doctor, if you'd like to -- to pull up</p> <p>14 Hamilton, you may do that.</p> <p>15 MS. BROWN:</p> <p>16 Q And we'll certainly give you time to do</p> <p>17 that, Doctor.</p> <p>18 Sitting here today, do you recall that</p> <p>19 to be the conclusion of Hamilton?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 You don't -- if you need to see the --</p> <p>23 MS. BROWN:</p> <p>24 Counsel --</p>	<p>1 Yes, you are. If he's asked to see the</p> <p>2 paper, he gets to look at the paper. Because</p> <p>3 this is not a situation where you can say, "Oh,</p> <p>4 I'll show it to you later," ask all these</p> <p>5 questions, try to get him to answer when he said</p> <p>6 I want to see the paper and review it. That's</p> <p>7 the way this works.</p> <p>8 MS. BROWN:</p> <p>9 Q Dr. Levy, can you answer the question</p> <p>10 without looking at the paper?</p> <p>11 MS. O'DELL:</p> <p>12 Would you repeat the question just to</p> <p>13 make sure we've got it?</p> <p>14 MS. BROWN:</p> <p>15 Yes. Would you please keep your</p> <p>16 objections to form in accordance with the federal</p> <p>17 rules?</p> <p>18 MS. O'DELL:</p> <p>19 My objections have been in accordance</p> <p>20 with the federal rules.</p> <p>21 MS. BROWN:</p> <p>22 Q Dr. Levy, my question to you was</p> <p>23 whether the Hamilton paper, the findings of the</p> <p>24 Hamilton paper show that chronic inflammation led</p>

8 (Pages 26 to 29)

Shawn Levy, Ph.D.

Page 30	Page 32
<p>1 to neoplastic changes. Do you recall that</p> <p>2 question?</p> <p>3 A I do recall the question.</p> <p>4 Q Can you answer that question without</p> <p>5 looking at the paper?</p> <p>6 A I would need to look at the paper to</p> <p>7 accurately answer your question.</p> <p>8 Q Absolutely. Do you have a copy on your</p> <p>9 computer?</p> <p>10 A I do.</p> <p>11 Q Okay. We'll mark it, so we're all on</p> <p>12 the same page, as Exhibit 7.</p> <p>13 (DEPOSITION EXHIBIT NUMBER 7</p> <p>14 WAS MARKED FOR IDENTIFICATION.)</p> <p>15 MS. BROWN:</p> <p>16 Q Here's a hard copy, Doctor, if that</p> <p>17 assists you.</p> <p>18 Doctor, looking at the Hamilton article</p> <p>19 that you have in front of you, does that refresh</p> <p>20 you that the authors found no association between</p> <p>21 the talc-induced changes and neoplasm?</p> <p>22 A No. Their -- their conclusions were</p> <p>23 that the talc-induced changes -- specifically</p> <p>24 fibrosis and the papillary changes -- did not</p>	<p>1 Q The Hamilton article does not support</p> <p>2 the theory that chronic inflammation leads to</p> <p>3 neoplastic changes in the ovary. Fair?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form.</p> <p>6 A The Hamilton article looked at an</p> <p>7 interval of one month, eighteen months, in a rat</p> <p>8 model. And, so, in the constraints of that</p> <p>9 particular experimental design and given the</p> <p>10 state of the art of the technology at the time,</p> <p>11 the authors did not conclude of a significant</p> <p>12 progression of ovarian cancer. But there's</p> <p>13 clearly limitations in both their experimental</p> <p>14 design and time course of the study to draw wide</p> <p>15 conclusions.</p> <p>16 MS. BROWN:</p> <p>17 Q The conclusions of the Hamilton</p> <p>18 article, Dr. Levy, do not support the hypothesis</p> <p>19 that chronic inflammation from talcum powder</p> <p>20 causes ovarian cancer. Would you agree?</p> <p>21 A I would not.</p> <p>22 Q The authors did not find that the</p> <p>23 inflammation seen in Hamilton led to neoplastic</p> <p>24 changes. True?</p>
Page 31	Page 33
<p>1 appear to be a reaction to talc, but they -- I</p> <p>2 don't see the specific inclusion that you asked</p> <p>3 in the question regarding neoplasm.</p> <p>4 Q I'm looking at page 103, Doctor, the</p> <p>5 first full paragraph that begins "no evidence."</p> <p>6 You with me?</p> <p>7 A One moment. "No evidence of cellular,"</p> <p>8 that paragraph?</p> <p>9 Q Yes.</p> <p>10 And, for the record, that paragraph</p> <p>11 reads, "No evidence of cellular atypia or mitotic</p> <p>12 activity was seen in the nonpapillary areas of</p> <p>13 the surface epithelium of the injected ovaries</p> <p>14 and in no ovary was there any evidence of frank</p> <p>15 neoplasia."</p> <p>16 Correct?</p> <p>17 A It does read that way, yes.</p> <p>18 Q And that was a conclusion of the</p> <p>19 Hamilton article. Correct?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 A That was an observation of the Hamilton</p> <p>23 article.</p> <p>24 MS. BROWN:</p>	<p>1 A The authors did not report observing</p> <p>2 neoplastic change over the time course of the</p> <p>3 given study.</p> <p>4 Q Doctor, I'm handing you the report that</p> <p>5 you've served in this case, which we'll mark as</p> <p>6 Exhibit 2.</p> <p>7 (DEPOSITION EXHIBIT NUMBER 2</p> <p>8 WAS MARKED FOR IDENTIFICATION.)</p> <p>9 MS. BROWN:</p> <p>10 Q And I'd like you to -- I'd like to</p> <p>11 direct you to page 14. I'd like to direct your</p> <p>12 attention to the last paragraph of -- the last</p> <p>13 sentence -- excuse me -- of the second full</p> <p>14 paragraph that begins "additional studies."</p> <p>15 Do you see that sentence, Doctor?</p> <p>16 A What's the beginning of that paragraph</p> <p>17 so I make sure I'm looking at the right one?</p> <p>18 Q Sure. I'd like to direct you on page</p> <p>19 14 of your report to the second full paragraph</p> <p>20 that begins "In addition to epidemiologic</p> <p>21 evidence."</p> <p>22 Do you see that?</p> <p>23 A I do.</p> <p>24 Q The last paragraph, or the last</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 34</p> <p>1 sentence of that paragraph in your report reads, 2 "Additional studies have also shown the effects 3 of talc on the immune response." 4 Do you see that sentence? 5 A I do. 6 Q And you cite the Hamilton article for 7 that proposition that we were just reviewing? 8 A Uh-huh. 9 Q True? 10 A True. 11 Q And the talc effects on the immune 12 response that were shown in Hamilton were not 13 effects that the authors observed led to 14 neoplastic changes. Correct? 15 MS. O'DELL: 16 Object to the form. 17 A I'm sorry. I'm not sure I understand 18 your question. 19 MS. BROWN: 20 Q Sure. 21 A Are you asking, if I could clarify, are 22 you -- are you asking if Hamilton is an 23 appropriate reference for the effects of talc on 24 the immune response or are you asking if</p>	<p style="text-align: right;">Page 36</p> <p>1 hypothesis that chronic inflammation leads to 2 cancer in animals. Right? 3 A The -- 4 MS. O'DELL: 5 Object to the form. 6 A The -- those two references were not 7 included in the report to provide the opinion or 8 conclusions that you just described. 9 MS. BROWN: 10 Q Because you know, Doctor, that there's 11 not a single animal study that shows that talc 12 causes changes in animals that leads to cancer; 13 right? 14 MS. O'DELL: 15 Object to the form. 16 A Could you -- could you phrase that 17 question again? Sorry. 18 MS. BROWN: 19 Q There is not a single animal study, 20 Doctor, that supports the opinion that chronic 21 inflammation caused by talc causes ovarian 22 cancer. Is that correct? 23 MS. O'DELL: 24 Object to the form.</p>
<p style="text-align: right;">Page 35</p> <p>1 Hamilton's an appropriate reference for something 2 else? 3 Q In your report, you state that studies, 4 such as Hamilton, have shown effects of talc on 5 the immune response. Correct? 6 A That is correct. 7 Q And you said Hamilton as one of the 8 articles that supports that proposition. True? 9 A Of the immune response, that's true. 10 Q Okay. The immune response that was 11 observed in Hamilton was not an immune response 12 that led to cancer. Right? 13 A As -- as I stated earlier, on the time 14 course of the Hamilton study, the authors did not 15 report specifically to neoplastic change in the 16 rat or conclude or make that conclusion, nor did 17 they conclude that that was not a possibility 18 either. 19 Q And on page 14 of your report you have 20 two additional cites for that proposition; 21 correct? 22 A Correct. 23 Q And you know, Doctor, that neither of 24 those cites, Keskin or NTP, support the</p>	<p style="text-align: right;">Page 37</p> <p>1 A In my review of the literature, there 2 are a number of animal studies that support the 3 opinions in the report regarding the biological 4 plausibility of talc leading to or contributing 5 to neoplastic change. 6 MS. BROWN: 7 Q Are you aware of any animal studies, 8 Doctor, that show talc causing chronic 9 inflammation in animals that leads to neoplastic 10 or cancerous changes in the animals? 11 MS. O'DELL: 12 Object to the form. Compound. 13 A There is one 1971 study that I'm aware 14 of. I would have to review to remember the 15 author. That was an earlier seminal -- or a 16 earlier study that described the role of talcum 17 powder and the inflammatory change within the 18 ovary. 19 MS. BROWN: 20 Q Who's the author of that study, Doctor? 21 A I'm trying to think of where I have 22 that reference. 23 Q Why don't we put that to the side and 24 at a break we'll see if we can find that article</p>

10 (Pages 34 to 37)

Shawn Levy, Ph.D.

Page 38	Page 40
<p>1 and then we can take a look at it. Okay?</p> <p>2 A Uh-huh.</p> <p>3 Q Okay. Getting back, then, Doctor, to</p> <p>4 what we had marked as Exhibit 6, which is the</p> <p>5 Taher paper, fair to say you reviewed that paper</p> <p>6 after your report was submitted in this case?</p> <p>7 A Yes.</p> <p>8 Q Okay. And did you notice throughout</p> <p>9 Taher's paper he makes reference to a number of</p> <p>10 supplemental materials?</p> <p>11 A Not specifically.</p> <p>12 Q Are you in receipt from plaintiffs'</p> <p>13 counsel of those supplemental materials?</p> <p>14 A I'd have to -- you'd have to give me a</p> <p>15 specific example, and I would be able to answer</p> <p>16 you.</p> <p>17 Q So, throughout the paper, the authors</p> <p>18 make reference to a set of supplemental materials</p> <p>19 that support their opinions. Do you recall that?</p> <p>20 A I certainly recall the reference</p> <p>21 materials to support their opinion. Whether they</p> <p>22 were supplemental or otherwise, that doesn't</p> <p>23 stand out to me.</p> <p>24 Q Okay. And I'm not trying to be tricky.</p>	<p>1 A And I have those on the -- available</p> <p>2 electronically.</p> <p>3 Q Okay. Were you provided with completed</p> <p>4 versions of all the plaintiff experts in the MDL</p> <p>5 proceeding?</p> <p>6 A I can't speak to whether it was all,</p> <p>7 but I have been provided with several.</p> <p>8 Q Will you list for me the expert reports</p> <p>9 you've been provided with?</p> <p>10 A Sure.</p> <p>11 Q Thank you.</p> <p>12 A There are four on -- on this drive,</p> <p>13 three -- I'm sorry. Two. Crowley and Longo.</p> <p>14 Q Two reports from Dr. Crowley and two</p> <p>15 reports from Dr. Longo?</p> <p>16 MS. O'DELL:</p> <p>17 I don't think that's what he said.</p> <p>18 A No. I think there are two, two expert</p> <p>19 reports, one from Dr. Crowley and one from</p> <p>20 Dr. Longo.</p> <p>21 MS. BROWN:</p> <p>22 Q Okay. And the date of the Crowley</p> <p>23 report, please?</p> <p>24 A The -- according to the file, the</p>
Page 39	Page 41
<p>1 I just want to know if you have those materials,</p> <p>2 and, if so, I'm gonna request production of them.</p> <p>3 A No. I -- I -- I don't believe that I</p> <p>4 have the full list of reference -- of literature</p> <p>5 cited from that -- from this paper --</p> <p>6 Q Okay.</p> <p>7 A -- now --</p> <p>8 Q Now, Taher --</p> <p>9 A -- but I'd have to check.</p> <p>10 Q Sorry.</p> <p>11 The Taher paper did not inform your --</p> <p>12 the opinions contained in your report dated</p> <p>13 November of 2018; correct?</p> <p>14 A Correct, as written.</p> <p>15 Q Okay. Are there any additional</p> <p>16 documents that either you or your counsel have</p> <p>17 brought with you here today in response to</p> <p>18 Exhibit 1, the Notice of Deposition?</p> <p>19 A So I'm not sure how to answer that</p> <p>20 accurately, but I would say there's a -- I've</p> <p>21 been provided with -- since the completion of my</p> <p>22 report, I've been provided with reports from</p> <p>23 other experts in the -- in the case.</p> <p>24 Q Okay.</p>	<p>1 date -- the modified date is November 28, 2018.</p> <p>2 Q And --</p> <p>3 A Whether that was the written date, I --</p> <p>4 I don't know.</p> <p>5 Q And the Longo report, do you know the</p> <p>6 date of that?</p> <p>7 A It is listed as August 2nd, 2017, in</p> <p>8 the title. And then there's a -- sorry. There's</p> <p>9 a second Longo report, 2018, which has a</p> <p>10 November 28, 2018, date. So my -- my apologies.</p> <p>11 To correct, there are two expert reports from</p> <p>12 Dr. Longo.</p> <p>13 Q Got it.</p> <p>14 MS. O'DELL:</p> <p>15 So when you were talking about --</p> <p>16 MS. BROWN:</p> <p>17 Counsel, no. Huh-uh. No. We -- I'm</p> <p>18 gonna ask questions, and he's gonna answer. We</p> <p>19 are not going to have you testify. You are not</p> <p>20 to testify about the expert reports.</p> <p>21 MS. O'DELL:</p> <p>22 I'm not gonna --</p> <p>23 You asked him what the date of the</p> <p>24 report was.</p>

11 (Pages 38 to 41)

Shawn Levy, Ph.D.

Page 42	Page 44
<p>1 MS. BROWN: 2 He -- then he will answer, counsel. 3 You can't testify. 4 MS. O'DELL: 5 He gave you the date of the file -- the 6 file date -- 7 MS. BROWN: 8 That's fine. 9 MS. O'DELL: 10 -- not the date -- 11 MS. BROWN: 12 On redirect, you are welcome to clean 13 up whatever you need to. But we're not gonna 14 have your testimony on the record about dates of 15 expert reports. 16 A So, looking at the report itself, the 17 date of the Longo report is November 14th, 2018. 18 MS. BROWN: 19 Q And were you provided -- 20 A The -- would you like the date of the 21 earlier report? 22 Q That would be terrific. 23 A It's August 2nd, 2017. 24 Q Great.</p>	<p>1 MS. BROWN: 2 Q How did you receive them? Was it email 3 or hard copy? 4 A Neither. They were made available 5 through a shared storage. 6 Q And would you have received an email 7 alerting you to their existence on a shared file? 8 MS. O'DELL: 9 Dr. Levy, communications between 10 counsel are -- are subject to the work product 11 privilege. 12 So to the degree you're asking him to 13 convey what was in a communication, then I'll 14 object to that and instruct you not to discuss 15 communications between counsel. 16 MS. BROWN: 17 Q Which the question does not ask for, 18 Doctor. 19 MS. O'DELL: 20 I believe it does. 21 MS. BROWN: 22 Q Here's what I want to know. Did you 23 rely on any other expert reports in forming your 24 opinions in this case?</p>
Page 43	Page 45
<p>1 Were you provided the two Longo reports 2 and the Dr. Crowley report by plaintiffs' 3 counsel? 4 A Yes. 5 Q Do you recall when? 6 A Not specifically. It was, obviously, 7 by their date, sometime after their completion. 8 So the Crowley report and the later 2018 Longo 9 report were sometime in November or December 10 2018. 11 There's -- I've also had an opportunity 12 to review a number of -- several other expert 13 reports which are not with me today. 14 Q Do you have a listing of the additional 15 expert reports you were provided with? 16 A I'd have to -- I could certainly -- I'd 17 have to provide it. I don't, off the top of my 18 head, recall all of them. There was probably 19 approximately a dozen. 20 Q Were all of the plaintiff expert 21 reports sent to you at once? 22 MS. O'DELL: 23 Object to the form. 24 A I'm not -- I'm not certain.</p>	<p>1 A To -- to my -- the content of my 2 report, no. 3 Q Did you receive the Crowley and two 4 Longo reports after you had already completed 5 your report in this case? 6 MS. O'DELL: 7 Object to the form. 8 A No. There was -- if I recall -- and 9 the -- at least the earlier Longo report -- and 10 I'd have to review the specifics -- at least the 11 earlier Longo report was reviewed and was 12 included in the content in the report. 13 And I would have to -- since the later 14 Longo report and then the final version of this 15 report were quite close together, I don't recall 16 if they overlapped or not. I'd have to review 17 the -- which references I used in here, which 18 will just take a moment. 19 So, yes, the -- I did include both 20 Longo reports. 21 Q The second Longo report was finalized 22 two days prior to your report. Is that right? 23 A Finalized, yes. 24 Q Did you see a draft of Longo's 2018</p>

12 (Pages 42 to 45)

Shawn Levy, Ph.D.

Page 46	Page 48
<p>1 report?</p> <p>2 A Yes. And the --</p> <p>3 Q And did you --</p> <p>4 A And as to when I saw the draft, I</p> <p>5 believe it was -- and it was sometime in the fall</p> <p>6 and/or when reports were being revised and</p> <p>7 expanded as more literature became available.</p> <p>8 Q Prior to Longo finalizing and signing</p> <p>9 his expert report in the MDL, you had access to a</p> <p>10 draft of that report; is that right?</p> <p>11 MS. O'DELL:</p> <p>12 Object to the form.</p> <p>13 A I can't speak to -- to that accurately.</p> <p>14 MS. BROWN:</p> <p>15 Q I thought you just testified you saw a</p> <p>16 version of the Longo 2018 report that was not</p> <p>17 final. Is that correct?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A I'd have to -- I'd have to review</p> <p>21 my -- the -- the literature that I used for the</p> <p>22 report to accurately answer your question.</p> <p>23 MS. BROWN:</p> <p>24 Q Well, your report doesn't say a draft,</p>	<p>1 Q Did you type the expert report that</p> <p>2 we've marked as Exhibit 2 yourself?</p> <p>3 A I did.</p> <p>4 Q Did you write all contents of Exhibit 2</p> <p>5 yourself?</p> <p>6 A I did.</p> <p>7 Q Were there parts of your report that</p> <p>8 you lifted from other published articles?</p> <p>9 MS. O'DELL:</p> <p>10 Object to the form.</p> <p>11 A Could you describe "lifted"?</p> <p>12 MS. BROWN:</p> <p>13 Q Did you take the words of other authors</p> <p>14 and put them in your expert report as Exhibit 2?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 A No. My -- my -- so my report is a</p> <p>18 review of the available literature at the time</p> <p>19 that the report was being developed. So, as</p> <p>20 such, it describes that -- that literature.</p> <p>21 As far as did I specifically copy words</p> <p>22 from other reports, no.</p> <p>23 MS. BROWN:</p> <p>24 Q Did you work with another plaintiff</p>
Page 47	Page 49
<p>1 and I'm wondering if you ever saw a non-finalized</p> <p>2 copy of the Longo report.</p> <p>3 A I didn't have an opportunity to compare</p> <p>4 the finalized Longo report to a -- what may be a</p> <p>5 draft or not to accurately answer your question</p> <p>6 if I saw a draft that was substantially different</p> <p>7 than what's referenced as the final.</p> <p>8 Q There were two days between Longo</p> <p>9 serving his report and you serving your report.</p> <p>10 Does that help orient you as to whether you saw a</p> <p>11 draft or you saw the final version?</p> <p>12 A Certainly possible I saw the final</p> <p>13 version.</p> <p>14 Q How many hours did you spend on your</p> <p>15 report in this case, Doctor?</p> <p>16 A The initial draft of the report? The</p> <p>17 initial writing of the report?</p> <p>18 Q In total, how many hours did you spend</p> <p>19 writing your report?</p> <p>20 A It was 20 hours initially, and then it</p> <p>21 would be -- it would be difficult to provide an</p> <p>22 accurate answer for the rest of that. I would</p> <p>23 say an additional few hours that I counted as</p> <p>24 revision.</p>	<p>1 expert on the report that we've marked as</p> <p>2 Exhibit 2?</p> <p>3 A I did not.</p> <p>4 Q Do you know who Dr. Zelikoff is?</p> <p>5 A The name's not familiar to me.</p> <p>6 Q Did you review a draft of</p> <p>7 Dr. Zelikoff's report before submitting your own?</p> <p>8 A I did not.</p> <p>9 Q Do you think that --</p> <p>10 A Not that I'm aware of.</p> <p>11 Q Do you have any explanation as to why a</p> <p>12 paragraph in your report is the same as a</p> <p>13 paragraph in Dr. Zelikoff's report?</p> <p>14 MS. O'DELL:</p> <p>15 Object to the form.</p> <p>16 A I -- without knowing -- without seeing</p> <p>17 the paragraph in both reports would be -- I can't</p> <p>18 comment.</p> <p>19 MS. BROWN:</p> <p>20 Q Let's mark as Exhibit 8 the expert</p> <p>21 report of Dr. Judith Zelikoff, Ph.D.</p> <p>22 (DEPOSITION EXHIBIT NUMBER 8</p> <p>23 WAS MARKED FOR IDENTIFICATION.)</p> <p>24 MS. BROWN:</p>

13 (Pages 46 to 49)

Shawn Levy, Ph.D.

Page 50	Page 52
<p>1 Q Is this something you've seen --</p> <p>2 Oh, sorry. Can I --</p> <p>3 It's okay, actually. It will flag it</p> <p>4 for you?</p> <p>5 Is this a report that you've seen</p> <p>6 before, Doctor?</p> <p>7 A I'll have to see it before I answer.</p> <p>8 Q I'm handing you what we've marked as</p> <p>9 Exhibit 8, which is the expert report of</p> <p>10 Dr. Judith Zelikoff. Is this one of the reports</p> <p>11 that you reviewed prior -- you reviewed at all?</p> <p>12 A I would have -- I would actually have</p> <p>13 to review my -- the literature that I reviewed</p> <p>14 in -- the totality of the literature that I</p> <p>15 reviewed, which I could answer that after a</p> <p>16 break, if necessary. But I don't recall,</p> <p>17 specifically recall, this report under</p> <p>18 Dr. Zelikoff's name. But it is certainly</p> <p>19 possible that I may have seen...</p> <p>20 Q Let's look at page 5 of your report,</p> <p>21 Doctor.</p> <p>22 A Okay.</p> <p>23 Q And why don't you put that side by side</p> <p>24 with page 20 of Dr. Zelikoff's report. And the</p>	<p>1 A They are --</p> <p>2 Q The next sentence --</p> <p>3 A Just one moment, please. I'm just</p> <p>4 making sure. Your question was are they exactly</p> <p>5 the same, and I'm just confirming if they're</p> <p>6 exactly the same.</p> <p>7 So, yes, I agree they're exactly the</p> <p>8 same.</p> <p>9 Q You have reviewed them and satisfied</p> <p>10 yourself that that -- those two sentences are</p> <p>11 exactly the same; correct?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form.</p> <p>14 A There's a single sentence in each</p> <p>15 report that is exactly the same. But important</p> <p>16 to comment that this single sentence is a -- is a</p> <p>17 basic biological premise of cancer, and, so,</p> <p>18 there's no surprise that two expert witnesses</p> <p>19 offering opinions on the role of -- or the</p> <p>20 biological plausibility or mechanisms of</p> <p>21 development of cancer would introduce a</p> <p>22 fundamental premise in the same manner.</p> <p>23 MS. BROWN:</p> <p>24 Q No surprise that you experts would have</p>
Page 51	Page 53
<p>1 paragraph in Dr. Zelikoff's report that I want to</p> <p>2 direct you to is the first full paragraph on</p> <p>3 page 20 that begins "Genetic mutations."</p> <p>4 Do you see that?</p> <p>5 A I do.</p> <p>6 Q And the paragraph of your report I want</p> <p>7 to direct you to is the paragraph on page 5 that</p> <p>8 begins "Both inherited."</p> <p>9 Do you see that?</p> <p>10 A I do.</p> <p>11 Q Okay. The first sentence of that</p> <p>12 paragraph in your report reads, "Both inherited</p> <p>13 and acquired gene -- and acquired gene mutations</p> <p>14 work together to cause cancer."</p> <p>15 Do you see that?</p> <p>16 A I do.</p> <p>17 Q The third sentence of the paragraph I</p> <p>18 directed you to in Dr. Zelikoff's report is</p> <p>19 identical and reads, "Both inherited and acquired</p> <p>20 gene mutations work together to cause cancer."</p> <p>21 Do you see that?</p> <p>22 A I do.</p> <p>23 Q Those two sentences are exactly the</p> <p>24 same, are they not?</p>	<p>1 one sentence that's the same? Is that what</p> <p>2 you're saying?</p> <p>3 MS. O'DELL:</p> <p>4 Objection. That's not what he said.</p> <p>5 Misrepresents his testimony.</p> <p>6 A I'm saying that both would -- both</p> <p>7 reports detail a fundamental aspect as they</p> <p>8 would -- based on the current understanding of</p> <p>9 the -- that both inherited and acquired gene</p> <p>10 mutations work in concert to cause cancer.</p> <p>11 MS. BROWN:</p> <p>12 Q Look at the next sentence on page 20 of</p> <p>13 Dr. Zelikoff's report. It reads as follows:</p> <p>14 "Even if one has inherited a genetic mutation</p> <p>15 that predisposes one to cancer," comma, "that</p> <p>16 doesn't mean he or she is certain to get cancer."</p> <p>17 Did I read that correctly?</p> <p>18 A You did.</p> <p>19 Q And let's go back to page 5 of your</p> <p>20 report. Skip ahead, if you would -- one, two,</p> <p>21 three -- four sentences to where you were and</p> <p>22 find the sentence that begins "Even."</p> <p>23 Are you with me?</p> <p>24 A I am.</p>

14 (Pages 50 to 53)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 54</p> <p>1 Q And your report at page 5 reads, "Even 2 if one has inherited a genetic mutation that 3 predisposes one to cancer," comma, "that doesn't 4 mean he or she is certain to get cancer." 5 Did I read that correctly? 6 A You did. 7 Q That's the exact same sentence we just 8 read in Dr. Zelikoff's report; correct? 9 A It is. 10 Q So now we have two sentences that are 11 exactly the same in your report and 12 Dr. Zelikoff's report. Correct? 13 MS. O'DELL: 14 Object to the form. 15 A You have two sentences that are written 16 the same but certainly not in precisely the same 17 context or organization in the total report. 18 MS. BROWN: 19 Q We have two sentences that are 20 word-for-word identical in two of the plaintiffs' 21 expert reports in this litigation. Is that fair? 22 MS. O'DELL: 23 Objection. Asked and answered. 24 A So reading your earlier question, you</p>	<p style="text-align: right;">Page 56</p> <p>1 identical to your report; correct? 2 A We have. 3 Q Do you have any explanation for why 4 that would be? 5 A I do. 6 Q What's that? 7 A That these -- each of these sentences 8 are describing basic introductory information 9 around the relationship between cancer and 10 genetic mutation. 11 Q And each of you described it with the 12 exact same words? 13 A Apparently so. 14 Q Let's keep going. 15 Page 20 of Dr. Zelikoff's report, 16 picking up where we left off, Dr. Zelikoff 17 writes: "The inherited gene mutation could 18 instead make one more likely to develop cancer 19 when exposed to certain cancer-causing 20 substances." 21 Do you see that? 22 A I do. 23 Q And let's go back to where we were in 24 your report, on page 5. "The inherited gene</p>
<p style="text-align: right;">Page 55</p> <p>1 asked, "Is that the same exact sentence we just 2 read in Dr. Zelikoff's report; correct?" And my 3 answer was "It is." And it remains the same. 4 Q Let's keep going. 5 Next sentence, at page 20 in 6 Dr. Zelikoff's report, states as follows: 7 "Rather," comma, "one or more additional gene 8 mutations may be needed to cause cancer." 9 Did I read that correctly? 10 A You did. 11 Q Let's go back to page 4 -- excuse me -- 12 page 5 of your report where we just were. And 13 you write: "Rather," comma, "one or more 14 additional gene mutations may be needed to cause 15 cancer." Correct? 16 A Correct. 17 Q That is the identical sentence from 18 Dr. Zelikoff's report. Correct? 19 A Starting with "Rather, one or more 20 additional gene mutations may be needed to cause 21 cancer." 22 Yes, correct. 23 Q So we now have identified three 24 sentences in Dr. Zelikoff's report that are</p>	<p style="text-align: right;">Page 57</p> <p>1 mutation could instead make one more likely to 2 develop cancer when exposed to a certain 3 cancer-causing substance." 4 Do you see that? 5 A I do. 6 Q And other than the tense in that last 7 sentence, they, too, are identical. Correct? 8 A So they're -- they're certainly similar 9 sentences, but that -- I believe the tense is an 10 important difference between them. 11 Again, as I stated, that these are 12 introductory and fundamental perspectives on 13 cancer and that, in this case, two expert 14 witnesses have summarized those things in a 15 similar fashion. 16 Q It doesn't strike you as odd that four 17 sentences are identical from two expert reports? 18 MS. O'DELL: 19 Object to the form. 20 A Four sentences are not identical. 21 MS. BROWN: 22 Q There's one small change in a tense. 23 That's it. Right, Doctor? 24 MS. O'DELL:</p>

15 (Pages 54 to 57)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 58</p> <p>1 Object to the form.</p> <p>2 A There -- there are -- there are three</p> <p>3 sentences which are, when considered</p> <p>4 individually, they are the same words. When you</p> <p>5 consider the -- now the group of those four</p> <p>6 sentences together between the two reports, they</p> <p>7 are clearly different organization with</p> <p>8 significantly more information between those</p> <p>9 identical sentences in one or the other.</p> <p>10 So the suggestion that they were -- one</p> <p>11 report was copied into the other, I would say it</p> <p>12 is equally interesting that they are more</p> <p>13 different than they are alike, other than the</p> <p>14 wording of three sentences.</p> <p>15 MS. BROWN:</p> <p>16 Q Did someone other than you write the</p> <p>17 sentences we've just been looking at in your</p> <p>18 report?</p> <p>19 A No.</p> <p>20 Q Did you consult the Mayo Clinic's</p> <p>21 website in connection with writing your report?</p> <p>22 A I don't believe so.</p> <p>23 Q Do you consider the Mayo Clinic's</p> <p>24 website to be authoritative -- an authoritative</p>	<p style="text-align: right;">Page 60</p> <p>1 Q -- next to your report, which remains</p> <p>2 Exhibit 2. And I will direct you to the second</p> <p>3 page of the Mayo Clinic printout, the section</p> <p>4 titled "Causes."</p> <p>5 Are you with me?</p> <p>6 A Second page.</p> <p>7 Q Double-sided. Flip it over.</p> <p>8 A Yes.</p> <p>9 Q Okay. And I'll direct you to page 3 of</p> <p>10 your report entitled "The Role of Gene Mutations</p> <p>11 in the Development of Cancer."</p> <p>12 A Uh-huh.</p> <p>13 Q Starting with Exhibit 9, the Mayo</p> <p>14 Clinic website, under a section entitled</p> <p>15 "Causes," the Mayo Clinic writes, "Cancer is</p> <p>16 caused by changes" -- parentheses --</p> <p>17 "(mutations) to the DNA within cells."</p> <p>18 Do you see that?</p> <p>19 A I do.</p> <p>20 Q And, looking at page 3 of your report,</p> <p>21 Doctor, that same sentence or sentence fragment</p> <p>22 appears in the first sentence: "Cancer is caused</p> <p>23 by changes" -- parentheses -- "(mutations) to the</p> <p>24 DNA within cells."</p>
<p style="text-align: right;">Page 59</p> <p>1 source, in your view?</p> <p>2 MS. O'DELL:</p> <p>3 Object to the form.</p> <p>4 A I have no basis for that opinion. I --</p> <p>5 I haven't reviewed the Mayo Clinic website to</p> <p>6 determine that.</p> <p>7 (DEPOSITION EXHIBIT NUMBER 9</p> <p>8 WAS MARKED FOR IDENTIFICATION.)</p> <p>9 MS. BROWN:</p> <p>10 Q Handing you, Doctor, what we've marked</p> <p>11 as Exhibit 9 to your deposition, which is a</p> <p>12 printout from the Mayo Clinic website entitled</p> <p>13 "Cancer."</p> <p>14 A Uh-huh.</p> <p>15 Q I'll hand it to you. And let me know</p> <p>16 if this is something that you've ever seen</p> <p>17 before.</p> <p>18 A Not that I recall.</p> <p>19 Q Did you take any language from the Mayo</p> <p>20 Clinic website to use in your report?</p> <p>21 A No.</p> <p>22 Q Let's take a -- I want you to put the</p> <p>23 Mayo Clinic, which we've marked as Exhibit 9 --</p> <p>24 A Uh-huh.</p>	<p style="text-align: right;">Page 61</p> <p>1 Correct?</p> <p>2 MS. O'DELL:</p> <p>3 Object to the form.</p> <p>4 A Say your question again. Are you</p> <p>5 asking --</p> <p>6 MS. BROWN:</p> <p>7 Q It's the same; right, Doctor?</p> <p>8 MS. O'DELL:</p> <p>9 Object to the form.</p> <p>10 A There are eight words or ten words that</p> <p>11 are the same in this first sentence, again, both</p> <p>12 describing some of the fundamental premise of</p> <p>13 cancer and its -- in its description.</p> <p>14 MS. BROWN:</p> <p>15 Q Let's go to the second sentence in the</p> <p>16 Mayo Clinic website, which reads, "The DNA inside</p> <p>17 a cell is packaged into a large number of</p> <p>18 individual genes, each of which contains a set of</p> <p>19 instructions telling the cell what functions to</p> <p>20 perform," comma, "as well as how to grow and</p> <p>21 divide."</p> <p>22 Do you see that?</p> <p>23 A I do.</p> <p>24 Q And a nearly identical version of that</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 62</p> <p>1 sentence appears in your report at page 3 where</p> <p>2 you state, "The DNA that makes up our genetic</p> <p>3 code is organized into a large number of</p> <p>4 individual genes, each of which contains a</p> <p>5 specific subset of instructions telling the cell</p> <p>6 what functions to perform," comma, "as well as</p> <p>7 how to grow and divide."</p> <p>8 Do you see that?</p> <p>9 A I do.</p> <p>10 Q Do you notice that nearly all the words</p> <p>11 are the same as the Mayo Clinic's?</p> <p>12 MS. O'DELL:</p> <p>13 Objection to form.</p> <p>14 A I, again -- we -- we have another</p> <p>15 example of similar language describing</p> <p>16 introductory and fundamental aspects surrounding</p> <p>17 the basics of cancer biology.</p> <p>18 MS. BROWN:</p> <p>19 Q Back to the Mayo Clinic next sentence.</p> <p>20 Quote: "Errors in the instructions can cause the</p> <p>21 cell to stop its normal function and may allow a</p> <p>22 cell to become cancerous."</p> <p>23 Do you see that?</p> <p>24 A I do.</p>	<p style="text-align: right;">Page 64</p> <p>1 subparagraph titled "Loss of DNA Repair."</p> <p>2 Are you with me?</p> <p>3 A Yes.</p> <p>4 Q I'm gonna read you two sentences from</p> <p>5 the Mayo Clinic. Tell me if I read them</p> <p>6 correctly.</p> <p>7 "DNA repair genes look for errors in a</p> <p>8 cell's DNA and make corrections. A mutation in a</p> <p>9 DNA repair gene may mean that other errors aren't</p> <p>10 corrected, leading cells to become cancerous."</p> <p>11 Do you see those two sentences, Doctor?</p> <p>12 A I do.</p> <p>13 Q Those are two sentences written by the</p> <p>14 folks who produce the Mayo Clinic's website;</p> <p>15 correct?</p> <p>16 A I -- I have no knowledge of who wrote</p> <p>17 that.</p> <p>18 Q The same two sentences appear in your</p> <p>19 report on page 4. Quote: "DNA repair genes look</p> <p>20 for errors in a cell's DNA and make corrections.</p> <p>21 A mutation in a DNA repair gene may mean that</p> <p>22 other errors aren't corrected, leading cells to</p> <p>23 become cancerous."</p> <p>24 Do you see that?</p>
<p style="text-align: right;">Page 63</p> <p>1 Q Back to your report at page 3. An</p> <p>2 identical sentence: "Errors in the instruction</p> <p>3 can cause the cell to stop its normal function</p> <p>4 and may allow a cell to become cancerous."</p> <p>5 Do you see that?</p> <p>6 A I do.</p> <p>7 Q Does that strike you as strange?</p> <p>8 MS. O'DELL:</p> <p>9 Object to the form.</p> <p>10 A Strange in what way?</p> <p>11 MS. BROWN:</p> <p>12 Q That your expert report in this</p> <p>13 litigation contains identical sentences to the</p> <p>14 Mayo Clinic's website.</p> <p>15 MS. O'DELL:</p> <p>16 Objection. Misstates the report.</p> <p>17 A I -- I don't find it surprising in the</p> <p>18 least.</p> <p>19 MS. BROWN:</p> <p>20 Q Let's turn to page 4 of your report,</p> <p>21 please. And I'll direct you to the final bullet</p> <p>22 on the same page of the Mayo Clinic website you</p> <p>23 were just looking at. The section of your report</p> <p>24 on page 4 I'd like to direct you to is the</p>	<p style="text-align: right;">Page 65</p> <p>1 A I do.</p> <p>2 Q Those two sentences are identical in</p> <p>3 the Mayo Clinic's website and your report. True?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form.</p> <p>6 A Again, we have fund- -- basic</p> <p>7 information that provides an introductory</p> <p>8 description of the basics of cancer which is used</p> <p>9 as -- as an inform- -- informatory foundation for</p> <p>10 latter opinions in the report but is not germane</p> <p>11 to the -- to the opinion in my report.</p> <p>12 And, again, as stated before, that</p> <p>13 succinct fundamental information regarding cancer</p> <p>14 biology in two sources that state things</p> <p>15 succinctly and clearly in layman's language</p> <p>16 are -- are similar or even identical, again, does</p> <p>17 not surprise me.</p> <p>18 MS. BROWN:</p> <p>19 Q We read at least four sentences that</p> <p>20 are identical to the Mayo Clinic. Would you</p> <p>21 agree?</p> <p>22 MS. O'DELL:</p> <p>23 Objection to form. The sentences are</p> <p>24 not identical.</p>

17 (Pages 62 to 65)

Shawn Levy, Ph.D.

Page 66	Page 68
<p>1 MS. BROWN: 2 Counsel, form. 3 A There are some similar -- there are 4 some similarly stated sentences that 5 you're -- that you've taken out of context in 6 both cases to find them identical. So I -- I 7 agree that they're identical, but, again, 8 don't -- don't necessarily am surprised since I 9 have no knowledge of where the information from 10 the Mayo website was taken from. 11 MS. BROWN: 12 Q You agree a number of sentences in your 13 report are identical to a number of sentences on 14 the Mayo Clinic's website. True? 15 MS. O'DELL: 16 Object to the form. 17 A No. I agree that they're -- I don't 18 agree. There are specific wordings that are the 19 same. 20 MS. BROWN: 21 Q Doctor, do you not agree that a number 22 of the sentences we just read are identical to a 23 number of sentences that appear on the Mayo 24 Clinic's website?</p>	<p>1 from our conversation to comment on those. 2 MS. BROWN: 3 Q You have it right in front of you. We 4 just looked at them. 5 A We did. 6 Q Right? 7 A Yes. 8 Q You recall reading a number of 9 sentences in the Mayo Clinic website that match 10 word for word a number of sentences in your 11 report. True? 12 MS. O'DELL: 13 Object to the form. 14 A We've -- we've read information that 15 is -- that is similar between the two documents. 16 And, as answered, given the, again, basic 17 fundamental introduction in lay language for 18 these concepts, it is no surprise that it's the 19 same. 20 MS. BROWN: 21 Q You're not surprised to find identical 22 sentences in your report and Dr. Zelickoff's 23 report? 24 A I'm not surprised.</p>
Page 67	Page 69
<p>1 MS. O'DELL: 2 Object to the form. 3 A I think we've -- we've specifically 4 gone over those individually and answered those 5 questions. 6 MS. BROWN: 7 Q And you'll agree the sentences are 8 identical? 9 MS. O'DELL: 10 Object to the form. 11 A Again, I -- I've answered -- I've 12 answered those when we went through them 13 individually. 14 MS. BROWN: 15 Q Well, I want you to answer my question 16 now. 17 You'll agree we've looked at a number 18 of sentences that are identical in your report to 19 the information on the Mayo Clinic's website; 20 correct? 21 MS. O'DELL: 22 Object to the form. Misstates his 23 testimony. 24 A I'd have to go back to the transcript</p>	<p>1 MS. O'DELL: 2 Object to the form. 3 MS. BROWN: 4 Q You are not surprised to find identical 5 sentences in your report and the Mayo Clinic? 6 MS. O'DELL: 7 Objection to form. Asked and answered. 8 A No. I -- I've answered that. 9 MS. BROWN: 10 Q You need to answer it again. 11 Are you -- 12 A I'm not surprised. 13 Q -- surprised? 14 Did you consult Wikipedia in writing 15 your expert report? 16 A I don't recall. 17 Q Do you think it's possible you might 18 have looked at Wikipedia when writing your expert 19 report in this litigation? 20 A I've -- I've looked -- I've looked at a 21 large number of sources in published literature 22 and others. 23 Q Did one of those sources include 24 Wikipedia?</p>

18 (Pages 66 to 69)

Shawn Levy, Ph.D.

Page 70	Page 72
<p>1 A I don't recall.</p> <p>2 Q Do you consider Wikipedia to be a</p> <p>3 scientifically reliable source?</p> <p>4 A What do you mean by scientifically</p> <p>5 reliable.</p> <p>6 Q Do you understand the concept of</p> <p>7 scientific reliability when answering a</p> <p>8 scientific question?</p> <p>9 MS. O'DELL:</p> <p>10 Object to the form.</p> <p>11 A Again, you'd have to -- that's -- you'd</p> <p>12 have to explain your -- what scientific</p> <p>13 reliability means in the context of your</p> <p>14 question.</p> <p>15 MS. BROWN:</p> <p>16 Q What does it mean to you?</p> <p>17 A Scientific reliability? In general</p> <p>18 terms, it would mean information that comes from</p> <p>19 a peer-reviewed source.</p> <p>20 Q And Wikipedia is not peer-reviewed;</p> <p>21 correct?</p> <p>22 A Wikipedia generally reso- -- uses</p> <p>23 a -- is a summary of commonly -- at least in</p> <p>24 scientific terms, a number of peer-reviewed</p>	<p>1 And we'll mark a Wikipedia page as</p> <p>2 Exhibit 10.</p> <p>3 (DEPOSITION EXHIBIT NUMBER 10</p> <p>4 WAS MARKED FOR IDENTIFICATION.)</p> <p>5 MS. BROWN:</p> <p>6 Q I would like to direct you, Dr. Levy,</p> <p>7 to the first full paragraph in your expert report</p> <p>8 at page 7.</p> <p>9 A Uh-huh.</p> <p>10 Q Do you see that?</p> <p>11 A I do.</p> <p>12 Q And I want to direct your attention to</p> <p>13 the sentence in the middle of that paragraph that</p> <p>14 begins "BRCA1 combined."</p> <p>15 Do you see that?</p> <p>16 A Yes.</p> <p>17 MS. BROWN:</p> <p>18 Q And I want to, side by side with</p> <p>19 Wikipedia, direct your attention to the third</p> <p>20 full paragraph that begins, as well, "BRCA1</p> <p>21 combined."</p> <p>22 You with me?</p> <p>23 A I am.</p> <p>24 Q Wikipedia writes, "BRCA1 combines with</p>
Page 71	Page 73
<p>1 sources, but it is --</p> <p>2 So from a true peer-review perspective,</p> <p>3 Wikipedia actually is peer-reviewed in the sense</p> <p>4 that anyone can contribute and edit the</p> <p>5 information in Wikipedia.</p> <p>6 Q Including our kids; right?</p> <p>7 MS. O'DELL:</p> <p>8 Object to the form.</p> <p>9 A Possible.</p> <p>10 MS. BROWN:</p> <p>11 Q Anyone in the world could edit a</p> <p>12 Wikipedia page. True?</p> <p>13 A I believe so.</p> <p>14 Q Is it your testimony, Doctor, that</p> <p>15 information from Wikipedia is a reliable resource</p> <p>16 when answering a scientific question?</p> <p>17 A No, that is not my testimony. That is</p> <p>18 not my testimony, no.</p> <p>19 Q Do you -- do you think you used</p> <p>20 Wikipedia here in writing your report?</p> <p>21 A Again, I -- I -- I don't recall using</p> <p>22 Wikipedia specifically.</p> <p>23 Q Okay. Let's take a look at your report</p> <p>24 at page 7, Doctor.</p>	<p>1 other tumor suppressors, DNA damage sensors, and</p> <p>2 single transducers to form a large multi-subunit</p> <p>3 protein complex known as BRCA1-associated genome</p> <p>4 surveillance complex" -- parens --</p> <p>5 "BAC-" -- excuse me -- "(BASC)," end parens.</p> <p>6 Do you see that?</p> <p>7 A I do.</p> <p>8 Q Turning to your report, page 7, you</p> <p>9 write, "BRCA1 combines with other tumor</p> <p>10 suppressors," comma, "DNA damage sensors, and</p> <p>11 signal transducers to form a large multi-subunit</p> <p>12 protein complex known as the BRCA1-associated</p> <p>13 genome surveillance complex" -- parens --</p> <p>14 (BASC)."</p> <p>15 Correct?</p> <p>16 A That is correct.</p> <p>17 Q Those two sentences, Doctor, are</p> <p>18 identical.</p> <p>19 A It appears so, yes.</p> <p>20 Q Okay.</p> <p>21 A Except for a -- the reference included</p> <p>22 on the Wikipedia page is not included in my</p> <p>23 report.</p> <p>24 Q Wikipedia has cited a reference, and</p>

19 (Pages 70 to 73)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 74</p> <p>1 your sentence stands without a reference. Is</p> <p>2 that right?</p> <p>3 A That's right.</p> <p>4 Q Other than the footnote, the two</p> <p>5 sentences we just read are identical. True?</p> <p>6 A Both sentences state the same fact in</p> <p>7 the same way. So, similar to our earlier</p> <p>8 discussions, we've now seen a large collection of</p> <p>9 fundamental factual information with -- with</p> <p>10 accurate information from now a number of sources</p> <p>11 that are stated in similar ways through</p> <p>12 Wikipedia, other expert reports, and websites all</p> <p>13 about the fundamentals of cancer.</p> <p>14 Q The two sentences we just read, Doctor,</p> <p>15 are identical. Correct?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A We read one sentence in Wikipedia.</p> <p>19 MS. BROWN:</p> <p>20 Q And it is identical. True?</p> <p>21 A Yes. The wording is the same. With,</p> <p>22 of course, Wikipedia, as you already stated,</p> <p>23 being editable by anybody and can pull that</p> <p>24 content from anywhere, and it's the -- I'd have</p>	<p style="text-align: right;">Page 76</p> <p>1 Q I'm sorry. What did we mark the</p> <p>2 Coussens as? 12?</p> <p>3 A Twelve.</p> <p>4 Q That should have been 11.</p> <p>5 We have marked the Coussens' article</p> <p>6 now correctly as Exhibit 11, and I'll direct you</p> <p>7 to the last two sentences of the first full</p> <p>8 paragraph. Put that, if you would, Doctor, side</p> <p>9 by side with your report at page 9, sentence that</p> <p>10 begins "in contrast," both sentences that begin</p> <p>11 "in contrast."</p> <p>12 Are you with me?</p> <p>13 A I am.</p> <p>14 Q All right. So, in this published</p> <p>15 article, Ms. or Dr. Coussens writes, "In</p> <p>16 contrast, proliferating cells that sustain</p> <p>17 DNA" --</p> <p>18 MS. O'DELL:</p> <p>19 Excuse me, Alli. Sorry. Tell me, are</p> <p>20 you in the second paragraph?</p> <p>21 MS. BROWN:</p> <p>22 I'm on the end of the first full</p> <p>23 paragraph.</p> <p>24 MS. O'DELL:</p>
<p style="text-align: right;">Page 75</p> <p>1 to review -- I'd have to look to see what</p> <p>2 reference 16 in Wikipedia is. But it's certainly</p> <p>3 possible that I and Wikipedia summarized the same</p> <p>4 information from the same source.</p> <p>5 Q Let's go to page 9 of your report. One</p> <p>6 of the articles that you relied on is an article</p> <p>7 by Lisa Coussens and Zena Werb. Do you recall</p> <p>8 that?</p> <p>9 A That does sound familiar, but I'll have</p> <p>10 to verify.</p> <p>11 Q Handing you what we've marked as</p> <p>12 Exhibit 12 [sic] to your report, the Coussens and</p> <p>13 Werb article.</p> <p>14 (DEPOSITION EXHIBIT NUMBER 11</p> <p>15 WAS MARKED FOR IDENTIFICATION.)</p> <p>16 A Yes, this is a -- this is a review.</p> <p>17 This is an insight review article, which, similar</p> <p>18 to my report, is likely consolidating information</p> <p>19 from the research knowledge.</p> <p>20 MS. BROWN:</p> <p>21 Q I'd like to direct you to the last two</p> <p>22 sentences of Exhibit 10, the Coussens' article,</p> <p>23 the last two sentences in the first paragraph.</p> <p>24 A Exhibit 10 or 12?</p>	<p style="text-align: right;">Page 77</p> <p>1 Sorry. I thought you were in the first</p> <p>2 full paragraph.</p> <p>3 MS. BROWN:</p> <p>4 Begins "In contrast."</p> <p>5 MS. O'DELL:</p> <p>6 Okay.</p> <p>7 MS. BROWN:</p> <p>8 And we have that side by side with</p> <p>9 Dr. Levy's report, page 9, the paragraph that</p> <p>10 also begins "In contrast."</p> <p>11 MS. O'DELL:</p> <p>12 Thank you.</p> <p>13 MS. BROWN:</p> <p>14 Q Dr. Coussens writes, "In contrast,</p> <p>15 proliferating cells that sustain DNA damage</p> <p>16 and/or mutagenic assault" -- parens -- "(for</p> <p>17 example, initiated cells), continue to</p> <p>18 proliferate in microenvironments rich in</p> <p>19 inflammatory cells and growth/survival factors</p> <p>20 that support their growth."</p> <p>21 Do you see that sentence?</p> <p>22 A I do.</p> <p>23 Q The next sentence reads, "In a sense,"</p> <p>24 comma, "tumors act as wounds that fail to heal."</p>

20 (Pages 74 to 77)

Shawn Levy, Ph.D.

Page 78	Page 80
<p>1 See that?</p> <p>2 A I do.</p> <p>3 Q Directing your attention to page 9 of</p> <p>4 your report, Doctor, you write, "In contrast,"</p> <p>5 comma, "proliferating cells that sustain DNA</p> <p>6 damage and/or mutagenic insult -- parens -- "(for</p> <p>7 example," comma, "initiated cells)," end paren,</p> <p>8 "continue to proliferate in microenvironments</p> <p>9 rich in inflammatory cells and growth/survival</p> <p>10 factors that support their growth," period. "In</p> <p>11 a sense, tumors act as wounds that fail to heal."</p> <p>12 Do you see that?</p> <p>13 A I do.</p> <p>14 Q Except for one word, Doctor, those two</p> <p>15 sentences, including the slashes and the</p> <p>16 parentheses, are identical. Correct?</p> <p>17 MS. O'DELL:</p> <p>18 Object to the form.</p> <p>19 A Those two sentences are similar.</p> <p>20 MS. BROWN:</p> <p>21 Q Except for one word, those two</p> <p>22 sentences are identical. True?</p> <p>23 MS. O'DELL:</p> <p>24 Object to the form. Asked and</p>	<p>1 Q My question, Doctor, was: Except for</p> <p>2 one word, the two sentences we just read from</p> <p>3 Coussens are identical to the two sentences in</p> <p>4 your report. Is that correct?</p> <p>5 MS. O'DELL:</p> <p>6 Object to the form.</p> <p>7 A So, I -- as -- as stated, the two</p> <p>8 sentences are similar.</p> <p>9 MS. BROWN:</p> <p>10 Q Except for one word, they are</p> <p>11 identical. Is that correct?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form. He's asked --</p> <p>14 you've asked the question. He's answered your</p> <p>15 question.</p> <p>16 A Again, the two sentences are similar.</p> <p>17 MS. BROWN:</p> <p>18 Q Do you understand "identical," what</p> <p>19 "identical" means?</p> <p>20 A Yes. Exactly the same.</p> <p>21 Q Okay. Except for one word, those two</p> <p>22 sentences are exactly the same in the Coussens</p> <p>23 article and your report. True?</p> <p>24 MS. O'DELL:</p>
Page 79	Page 81
<p>1 answered.</p> <p>2 A Yeah. I'd certainly appreciate the</p> <p>3 similarity between the -- between the two. But</p> <p>4 that's -- again, as we've been discussing now for</p> <p>5 an extensive amount of time, in the introductory</p> <p>6 review content of the report --</p> <p>7 In fact, I reference the Coussens and</p> <p>8 Werb paper, so certainly it's not a surprise that</p> <p>9 wording is similar between them and used similar</p> <p>10 language to describe, again, these factual</p> <p>11 aspects of fundamental cancer biology, including</p> <p>12 similar references.</p> <p>13 MS. O'DELL:</p> <p>14 Excuse me. My microphone is broken.</p> <p>15 VIDEOGRAPHER:</p> <p>16 It's still working. You're good. You</p> <p>17 can just lay it on the table and we'll fix it at</p> <p>18 a break.</p> <p>19 MS. O'DELL:</p> <p>20 And we've been going about an hour and</p> <p>21 13 minutes.</p> <p>22 MS. BROWN:</p> <p>23 I'm about to finish up this section.</p> <p>24 We'll take a break.</p>	<p>1 Object to the form. Asked and</p> <p>2 answered.</p> <p>3 A And we're -- we're saying the same</p> <p>4 thing in different ways, which is that the two</p> <p>5 sentences are similar, stating factual</p> <p>6 information about fundamental cancer biology and</p> <p>7 in two similar review articles.</p> <p>8 MS. BROWN:</p> <p>9 Q And the only difference is one word.</p> <p>10 Correct?</p> <p>11 A Two sentences are similar.</p> <p>12 Q My question was: The only difference</p> <p>13 is one word. True?</p> <p>14 A Let me review again to be sure that we</p> <p>15 would -- before answering.</p> <p>16 Taken out of context, those two</p> <p>17 sentences are similar.</p> <p>18 Q My question was, Doctor, the only</p> <p>19 difference is one word. Is that correct?</p> <p>20 MS. O'DELL:</p> <p>21 Objection to the form. Asked and</p> <p>22 answered.</p> <p>23 A You know, I think we've -- we've</p> <p>24 answered this a number of times, that the two</p>

21 (Pages 78 to 81)

Shawn Levy, Ph.D.

Page 82	Page 84
<p>1 sentences are different in their context and in</p> <p>2 terms of paragraph, but they are similar in</p> <p>3 structure and similar in wording.</p> <p>4 But, as you stated, with the exception</p> <p>5 of the -- so they're not. So in a language</p> <p>6 perspective, they're not identical. They're</p> <p>7 similar.</p> <p>8 MS. BROWN:</p> <p>9 Let's take a break.</p> <p>10 VIDEOGRAPHER:</p> <p>11 Going off -- going off the record. The</p> <p>12 time is 10:15 a m.</p> <p>13 (OFF THE RECORD.)</p> <p>14 VIDEOGRAPHER:</p> <p>15 We're back on the record. The time is</p> <p>16 10:25 a m.</p> <p>17 MS. BROWN:</p> <p>18 Q Doctor, I am handing you what I have</p> <p>19 marked as Deposition Exhibit 12 and 13. These</p> <p>20 are additional documents your counsel identified</p> <p>21 for us this morning as something you have seen</p> <p>22 since your report.</p> <p>23 (DEPOSITION EXHIBITS 12 AND 13</p> <p>24 WERE MARKED FOR IDENTIFICATION.)</p>	<p>1 A I have.</p> <p>2 Q Have you seen the reviewer comments</p> <p>3 referenced in Exhibit 13?</p> <p>4 A I have not seen the reviewer comments.</p> <p>5 Q Okay. Exhibit 13 does not inform the</p> <p>6 opinions of your report dated November of 2018.</p> <p>7 True?</p> <p>8 A Exhibit 13, being the letter, that is</p> <p>9 correct. It does not.</p> <p>10 Q Okay. And what's Exhibit 12?</p> <p>11 A Exhibit 12 appears to be a preprint</p> <p>12 version of the previously mentioned paper,</p> <p>13 "Molecular Basis Supporting the Association of</p> <p>14 Talcum Powder Use With Increased Risk of Ovarian</p> <p>15 Cancer," with the first author, Nicole Fletcher,</p> <p>16 and Dr. Saed is listed as the senior or</p> <p>17 corresponding author.</p> <p>18 Q Did the lawyers provide you with this</p> <p>19 manuscript, Doctor?</p> <p>20 A Yes, in a -- but that's -- yes, they</p> <p>21 did.</p> <p>22 Q Do you recall when you were provided</p> <p>23 with a copy of the manuscript by the plaintiffs'</p> <p>24 lawyers?</p>
Page 83	Page 85
<p>1 MS. BROWN:</p> <p>2 Q Would you tell us what those two</p> <p>3 exhibits are, please.</p> <p>4 A Exhibit -- Exhibit 13 is a printed copy</p> <p>5 of an email dated December 26th informing</p> <p>6 Dr. Saed that a manuscript --</p> <p>7 Is it helpful to identify the</p> <p>8 manuscript?</p> <p>9 -- titled "Molecular Basis Supporting</p> <p>10 the Association of Talcum Powder Use With</p> <p>11 Increased Risk of Ovarian Cancer," submitted to</p> <p>12 Reproductive Sciences, has been reviewed. The</p> <p>13 comments were included in the letter.</p> <p>14 Q Have you seen --</p> <p>15 A And I'm just reading the --</p> <p>16 Q Sure.</p> <p>17 A It -- it appears that the -- so,</p> <p>18 summarizing the letter, the manuscript has been</p> <p>19 reviewed, the comments from the reviewers were</p> <p>20 provided back, and the journal has informed</p> <p>21 Dr. Saed that they'll accept a revised document</p> <p>22 for potential publication.</p> <p>23 Q Have you seen Exhibit 13 prior to this</p> <p>24 morning?</p>	<p>1 A It was sometime in December toward --</p> <p>2 late in the year. The exact date, I'd have to</p> <p>3 review when it came in. And I believe it was --</p> <p>4 and the version you have here is a more formal</p> <p>5 preprint version from the -- from Manuscript</p> <p>6 Central, whereas the version I received</p> <p>7 was a -- it appeared to be more of a submission</p> <p>8 version.</p> <p>9 So commenting whether it's</p> <p>10 exact -- precisely the same content, I -- I</p> <p>11 wouldn't be able to say.</p> <p>12 Q Fair to say, though, Doctor, since you</p> <p>13 received the manuscript in December of 2018, the</p> <p>14 contents of the manuscript did not inform the</p> <p>15 expert report that you wrote in November of 2018;</p> <p>16 correct?</p> <p>17 A Actually, I would say the -- the -- I</p> <p>18 would not agree, from the perspective of Dr. Saed</p> <p>19 has a number of similar studies, as well as a</p> <p>20 number of abstracts that I had the opportunity to</p> <p>21 review that did inform some of the opinions in</p> <p>22 the report. Those same information and data were</p> <p>23 included in this manuscript and expanded upon</p> <p>24 actually significantly.</p>

22 (Pages 82 to 85)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 86</p> <p>1 So the basis of my opinion includes</p> <p>2 some of the information from this manuscript, but</p> <p>3 I -- but the report does not contain the totality</p> <p>4 of this.</p> <p>5 Q Right. Because the manuscript wasn't</p> <p>6 available to you until after you wrote your</p> <p>7 report. Right?</p> <p>8 A No, that's not the case. The -- the --</p> <p>9 the research, some of the research information</p> <p>10 from this study was available in abstract form,</p> <p>11 and -- and some -- I believe a preprint from</p> <p>12 Dr. Saed.</p> <p>13 So it was -- so it was available.</p> <p>14 Portions of it were available for the report.</p> <p>15 Q Other than the abstract, did you have</p> <p>16 access to an earlier version of what we've marked</p> <p>17 as Exhibit 12?</p> <p>18 A I can't accurately answer that without</p> <p>19 comparing them.</p> <p>20 Q Where do you have stored the earlier</p> <p>21 version that you're referring to?</p> <p>22 A Let's see if I -- what I have here.</p> <p>23 So, from Dr. Saed, I have a -- used a</p> <p>24 book chapter which describes some of his</p>	<p style="text-align: right;">Page 88</p> <p>1 Q Okay. And I'll ask if you'd be kind</p> <p>2 enough to do that at a break. Just let us know</p> <p>3 if you had access to something other than the</p> <p>4 abstract of Dr. Saed's 2018 report at the time</p> <p>5 you wrote your report. Fair enough?</p> <p>6 A I'll make a note.</p> <p>7 MS. O'DELL:</p> <p>8 Excuse me. Object to the form.</p> <p>9 Abstracts, not one.</p> <p>10 MS. BROWN:</p> <p>11 Q Dr. Levy, you are a Ph.D.; is that</p> <p>12 correct?</p> <p>13 A Correct.</p> <p>14 Q Okay. You are not an M.D.; correct?</p> <p>15 A That's correct.</p> <p>16 Q What's your Ph.D. in, sir?</p> <p>17 A Biochemistry and genetics.</p> <p>18 Q You're not an epidemiologist. Fair?</p> <p>19 A I am not.</p> <p>20 Q Okay. And the focus of your work at</p> <p>21 HudsonAlpha is on genome sequencing. Is that</p> <p>22 right?</p> <p>23 A No. The -- the -- genome sequencing is</p> <p>24 a tool that we apply in -- in the work of my</p>
<p style="text-align: right;">Page 87</p> <p>1 oxidative stress experiments that are also</p> <p>2 consistent with the information that's in the --</p> <p>3 in Exhibit 12, as well as some of his earlier</p> <p>4 review articles, and that's --</p> <p>5 Let me make sure I'm not missing</p> <p>6 anything from Fletcher, who's been...</p> <p>7 But, otherwise, the -- the experiments</p> <p>8 that were expanded upon in the formal manuscript</p> <p>9 were described in -- in abstract or, I should</p> <p>10 say, summarized form, meaning an abstract that</p> <p>11 included methods, results, and conclusions from</p> <p>12 Fletcher and colleagues in Dr. Saed's group.</p> <p>13 Q At the time you wrote your report, you</p> <p>14 had an abstract of the 2018 paper that we've</p> <p>15 marked as Exhibit 12; correct?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form. He said plural.</p> <p>18 A Yes. I had two abstracts and then</p> <p>19 possibly --</p> <p>20 I'd have to review when I received this</p> <p>21 preprint versus the final version of my report to</p> <p>22 see if they overlapped, if they're -- if I had an</p> <p>23 opportunity to review this or not.</p> <p>24 MS. BROWN:</p>	<p style="text-align: right;">Page 89</p> <p>1 laboratory and in my responsibilities at</p> <p>2 HudsonAlpha.</p> <p>3 Q HudsonAlpha has a team known as the</p> <p>4 Breakthrough Breast and Ovarian Cancer Team. Is</p> <p>5 that right?</p> <p>6 A I'm not familiar with that name.</p> <p>7 Q Okay.</p> <p>8 A There is a -- a group of faculty who</p> <p>9 have some funding related to breast and ovarian</p> <p>10 cancer. It's -- it's certainly possible that</p> <p>11 name was used in -- in press for some title.</p> <p>12 Q Since you're not familiar with that</p> <p>13 team, fair to say you're not a member of the</p> <p>14 Breakthrough Breast and Ovarian Cancer Team?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 A Again, I don't -- my involvement with</p> <p>18 breast and ovarian cancer at HudsonAlpha is</p> <p>19 specific to some projects. And whether or not I</p> <p>20 was named on that team, I -- I don't know.</p> <p>21 MS. BROWN:</p> <p>22 Q There are folks at HudsonAlpha,</p> <p>23 scientists and doctors at HudsonAlpha whose</p> <p>24 practice is devoted to studying ovarian cancer.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 90</p> <p>1 Correct?</p> <p>2 A No, that's not correct.</p> <p>3 Q Your practice is not devoted to ovarian</p> <p>4 cancer; correct?</p> <p>5 MS. O'DELL:</p> <p>6 Object to the form.</p> <p>7 A No. My -- my practice is not devoted</p> <p>8 to ovarian cancer. And -- but that was</p> <p>9 irrelevant to what I was asked to do in</p> <p>10 this -- in this particular case for</p> <p>11 the -- regarding the content of my report.</p> <p>12 MS. BROWN:</p> <p>13 Q I think I saw you've published one</p> <p>14 article regarding ovarian cancer over the course</p> <p>15 of your career. Is that right?</p> <p>16 A That sounds correct.</p> <p>17 Q You have not given any presentations</p> <p>18 regarding ovarian cancer. Is that true?</p> <p>19 A I would say that's accurate.</p> <p>20 Q You have not received any government</p> <p>21 funding to study ovarian cancer. True?</p> <p>22 A I received government funding to study</p> <p>23 breast and ovarian cancer -- this was in 2002,</p> <p>24 from the Department of Defense -- and then,</p>	<p style="text-align: right;">Page 92</p> <p>1 dating back to my early Ph.D. work, and those</p> <p>2 include cancer. So certainly the subject of</p> <p>3 inflammatory response in -- both chronic and</p> <p>4 acute, in controlling cancer has been a subject</p> <p>5 of my research for some time and certainly</p> <p>6 bridged into ovarian cancer as well as other</p> <p>7 cancer types.</p> <p>8 MS. BROWN:</p> <p>9 Q You've never published on chronic</p> <p>10 inflammation as a potential mechanism by which</p> <p>11 talcum powder causes ovarian cancer. Correct?</p> <p>12 A Not specific to talcum powder, no.</p> <p>13 Q You have never given a presentation on</p> <p>14 chronic inflammation as a mechanism for causing</p> <p>15 ovarian cancer at all; right?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A I'm thinking through my --</p> <p>19 I don't recall a specific presentation</p> <p>20 with regards to talcum powder and its role in</p> <p>21 ovarian cancer. As far as my discussions or</p> <p>22 presentations around the role of inflammation in</p> <p>23 cancer, including ovarian, it -- it is -- it is</p> <p>24 possible, but I can't think of a specific</p>
<p style="text-align: right;">Page 91</p> <p>1 subsequent to that, participated in at least one</p> <p>2 review for the Department of Defense in reviewing</p> <p>3 ovarian cancer research grants. So that is --</p> <p>4 And then my membership in the</p> <p>5 Vanderbilt Cancer Center as well as the</p> <p>6 University of Alabama Birmingham Comprehensive</p> <p>7 Cancer Center certainly have been involved in a</p> <p>8 number of projects across a diversity of cancer</p> <p>9 types, including ovarian and breast cancer.</p> <p>10 Q Prior to being hired by the plaintiffs'</p> <p>11 lawyers in this litigation, you had not</p> <p>12 investigated the potential mechanisms by which</p> <p>13 talcum powder could cause ovarian cancer. Is</p> <p>14 that fair?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 A Specific -- as in terms of a specific</p> <p>18 fundamental research project?</p> <p>19 MS. BROWN:</p> <p>20 Q At all.</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A So my research has included the role of</p> <p>24 inflammation and a number of biological processes</p>	<p style="text-align: right;">Page 93</p> <p>1 presentation.</p> <p>2 MS. BROWN:</p> <p>3 Q Okay. Since you've been hired by</p> <p>4 plaintiffs' lawyers, you have done some research</p> <p>5 into the potential role of inflammation and</p> <p>6 ovarian cancer. Is that right?</p> <p>7 MS. O'DELL:</p> <p>8 Object to the form.</p> <p>9 A Since -- since my -- what was requested</p> <p>10 of me from the plaintiffs' attorneys was to</p> <p>11 provide a review of the biological plausibility</p> <p>12 and a connection between talcum powder and</p> <p>13 inflammation and then discuss the relationship</p> <p>14 between inflammation and cancer.</p> <p>15 MS. BROWN:</p> <p>16 Q Okay. As I understand you, Dr. Levy,</p> <p>17 you were asked by the plaintiffs' lawyers to</p> <p>18 provide a review of the literature as it relates</p> <p>19 to the biological plausibility of talcum powder</p> <p>20 and ovarian cancer. Is that right?</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A No, that's not correct. What I was --</p> <p>24 I was asked to provide an opin- -- expert opinion</p>

24 (Pages 90 to 93)

Shawn Levy, Ph.D.

Page 94	Page 96
<p>1 on the biological plausibility of the mechanism</p> <p>2 that -- of the ability of exposure of talc and</p> <p>3 its constituent components to cause inflammation</p> <p>4 and/or cancer.</p> <p>5 MS. BROWN:</p> <p>6 Q Do you see those as two different</p> <p>7 things?</p> <p>8 A Yes.</p> <p>9 Q Okay. So you were asked to provide a</p> <p>10 mechanism by which talcum powder could cause</p> <p>11 cancer?</p> <p>12 A No, that's not correct.</p> <p>13 MS. O'DELL:</p> <p>14 Objection to form.</p> <p>15 MS. BROWN:</p> <p>16 Q Okay. Explain it to me.</p> <p>17 A I -- I was asked to provide a -- an</p> <p>18 opinion on the biological plausibility --</p> <p>19 Q Of talcum powder causing cancer?</p> <p>20 A -- of talcum powder leading to the</p> <p>21 biological changes necessary to cause cancer.</p> <p>22 Q Okay. As I understand what you just</p> <p>23 said, you were asked to re- -- to provide an</p> <p>24 opinion on the biological plausibility of talcum</p>	<p>1 some neurological diseases.</p> <p>2 So this was a similar review as -- of</p> <p>3 those topics when asked to examine the biological</p> <p>4 plausibility of a cause and effect; in this case,</p> <p>5 cause being exposure to talcum powder and effect</p> <p>6 being progression to cancer.</p> <p>7 Q Prior to being hired by the plaintiffs'</p> <p>8 lawyers, you had not considered the biological</p> <p>9 plausibility of talcum powder causing ovarian</p> <p>10 cancer. Correct?</p> <p>11 A No. I would say that's not true in</p> <p>12 isolation. And the reason I say that's not true</p> <p>13 is I had been aware of some of the literature and</p> <p>14 certainly some of the press that surrounded the</p> <p>15 suspected associations between talcum powder</p> <p>16 exposure and cancer. So I was familiar with the</p> <p>17 concept, but I had not at the time, until hired</p> <p>18 by the plaintiffs' attorney, spent a significant</p> <p>19 amount of time reviewing the literature and</p> <p>20 developing a written opinion as to that</p> <p>21 biological plausibility.</p> <p>22 Q You have not published your opinion</p> <p>23 contained in -- your opinions contained in the</p> <p>24 report that we marked as Exhibit 2. Is that</p>
Page 95	Page 97
<p>1 powder leading to biologic changes that are</p> <p>2 needed to cause cancer. Is that fair?</p> <p>3 MS. O'DELL:</p> <p>4 Object to the form.</p> <p>5 A So I was asked from -- by the attorneys</p> <p>6 to review the available literature across the</p> <p>7 spectrum of cancer and talcum powder and</p> <p>8 constituent literature to develop an opinion</p> <p>9 around the biological plausibility that exposure</p> <p>10 of -- exposure to talcum powder is</p> <p>11 biologically -- that there is a biologically</p> <p>12 plausible mechanism that that can cause cancer.</p> <p>13 MS. BROWN:</p> <p>14 Q Okay. And that is not something that</p> <p>15 you had done prior to being hired by the</p> <p>16 plaintiffs' lawyers. Fair?</p> <p>17 A Developing such an opinion?</p> <p>18 Q Correct.</p> <p>19 A Or -- or -- so writing such a report,</p> <p>20 no, that is not something I -- I had done prior</p> <p>21 to -- to this. My research has been primarily in</p> <p>22 data integration and the examination of</p> <p>23 mechanistic effects in cancer, rare disease,</p> <p>24 and -- and in diabetes specifically, as well as</p>	<p>1 correct?</p> <p>2 A That is correct.</p> <p>3 Q You have not presented the opinions</p> <p>4 contained in Exhibit 2 at any medical or</p> <p>5 scientific conference; correct?</p> <p>6 A That's correct.</p> <p>7 Q You have not disclosed the opinions</p> <p>8 contained in Exhibit 2 to any of your colleagues;</p> <p>9 correct?</p> <p>10 MS. O'DELL:</p> <p>11 Object to the form.</p> <p>12 A Not at this time, no. Considering I</p> <p>13 had -- I had just finalized the report a short</p> <p>14 time ago, I haven't had the opportunity to</p> <p>15 consider publication, presentation, or -- or</p> <p>16 discussion with colleagues.</p> <p>17 MS. BROWN:</p> <p>18 Q Do you plan to seek publication of the</p> <p>19 information contained in your report in Exhibit</p> <p>20 2?</p> <p>21 A I -- I haven't made a determination at</p> <p>22 this time. It's been a fascinating area to</p> <p>23 research. Certainly there's -- that would</p> <p>24 certainly be a future possibility.</p>

25 (Pages 94 to 97)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 98</p> <p>1 Q Does HudsonAlpha --</p> <p>2 First of all, what's your position at</p> <p>3 HudsonAlpha, Doctor?</p> <p>4 A So I'm a faculty investigator, which</p> <p>5 would be analogous to a faculty member at a</p> <p>6 research institution, similar to -- or I should</p> <p>7 take a step back and just --</p> <p>8 To be accurate, HudsonAlpha is a</p> <p>9 private nonprofit research institution, similar</p> <p>10 to Broad Institute, Stowers, et cetera. So we</p> <p>11 are academic in nature, meaning that most of our</p> <p>12 funding or the vast majority of our funding comes</p> <p>13 from grants and contracts. So that's why I say</p> <p>14 it's analogous to faculty at a research</p> <p>15 institution.</p> <p>16 My other responsibilities are the</p> <p>17 management and oversight of the production and</p> <p>18 research laboratories, so that provides us an</p> <p>19 opportunity to work with approximately 1200</p> <p>20 different laboratories from around the world in</p> <p>21 support of roughly 5,000 projects over the last</p> <p>22 nine and a half years. And that's -- it's</p> <p>23 provided a broad spectrum of activities and</p> <p>24 abilities to work in these types of projects.</p>	<p style="text-align: right;">Page 100</p> <p>1 or -- or -- or dispute whether or not those</p> <p>2 ovarian cancer or other cancer types may have had</p> <p>3 a relationship to talcum powder. So the short</p> <p>4 answer being I -- I don't have the information to</p> <p>5 answer that.</p> <p>6 MS. BROWN:</p> <p>7 Q HudsonAlpha has a Code of Ethics. Are</p> <p>8 you familiar with it?</p> <p>9 A Yes.</p> <p>10 Q Are you familiar with the financial</p> <p>11 disclosure requirements of HudsonAlpha?</p> <p>12 A I am.</p> <p>13 Q Have you complied with those in</p> <p>14 connection with your work as an expert witness</p> <p>15 for plaintiffs in this case?</p> <p>16 A I have.</p> <p>17 Q And tell us what you've done to comply</p> <p>18 with HudsonAlpha's Code of Ethics and financial</p> <p>19 disclosure requirements.</p> <p>20 A Their Code of Ethics and financial</p> <p>21 requirement is requirement to disclose any</p> <p>22 relationships that have a financial component</p> <p>23 over -- I don't recall the minimum amount, but it</p> <p>24 is -- it is fairly modest, hundreds of dollars.</p>
<p style="text-align: right;">Page 99</p> <p>1 And then I also oversee the clinical</p> <p>2 laboratories as well. And adult oncology is a</p> <p>3 major focus of that research. I currently lead</p> <p>4 the largest profiling effort in adult cancer in</p> <p>5 the nation, which involves 15 national cancer</p> <p>6 institutes. And ovarian cancer is a component of</p> <p>7 that research, although not the only cancer that</p> <p>8 we research in that -- in that's -- in that</p> <p>9 program.</p> <p>10 Q None of the 5,000 projects you just</p> <p>11 mentioned have dealt with talc. Is that fair?</p> <p>12 A That is fair.</p> <p>13 Q And none of the work at the clinical</p> <p>14 labs that you just mentioned have dealt with</p> <p>15 talc; correct?</p> <p>16 MS. O'DELL:</p> <p>17 Object.</p> <p>18 A I am -- I would say there's a</p> <p>19 statistical probability that some of the ovarian</p> <p>20 cancer samples that have been observed in the</p> <p>21 clinical laboratory may very well have</p> <p>22 been -- have come from patients exposed to talcum</p> <p>23 powder. But I have no direct knowledge of that,</p> <p>24 nor have we performed any testing to confirm</p>	<p style="text-align: right;">Page 101</p> <p>1 And that reporting requirement is the -- is -- is</p> <p>2 for the previous year, and it is due in July, I</p> <p>3 believe is the time frame, although I'd have to</p> <p>4 make sure. It's -- I know it's not the end of</p> <p>5 the calendar year. So on my next disclosure,</p> <p>6 this, of course, activity would be disclosed.</p> <p>7 In addition to that, via</p> <p>8 conversation -- regular review with the president</p> <p>9 of the institution, I provide a general report on</p> <p>10 consulting activities; for example, these</p> <p>11 activities.</p> <p>12 HudsonAlpha's policy is faculty members</p> <p>13 are allowed up to 20 percent of your time towards</p> <p>14 consulting activities that have a relationship to</p> <p>15 your research area, such as the evaluation of the</p> <p>16 biologically plausible mechanism of talc in</p> <p>17 ovarian cancer. So based on both the timing of</p> <p>18 the Code of Ethics with regards to the financial</p> <p>19 disclosure as well as the ad hoc reporting of</p> <p>20 consulting engagements with the president of the</p> <p>21 institution, I'm in compliance with the current</p> <p>22 policies of HudsonAlpha.</p> <p>23 Q The president of HudsonAlpha is aware</p> <p>24 of your opinions in this case?</p>

Shawn Levy, Ph.D.

Page 102	Page 104
<p>1 A I have not discussed my opinions</p> <p>2 specifically to this case with him; just the</p> <p>3 general knowledge that I was asked to participate</p> <p>4 as an expert witness. He didn't ask, and I</p> <p>5 didn't provide the content.</p> <p>6 Q No one at HudsonAlpha is aware of your</p> <p>7 opinion that talcum powder causes chronic</p> <p>8 inflammation which can cause ovarian cancer? Is</p> <p>9 that right?</p> <p>10 A I have -- I have not specifically</p> <p>11 shared the contents of the report or -- or my</p> <p>12 opinions widely at HudsonAlpha.</p> <p>13 Q Did you disclose last July that you had</p> <p>14 already been hired and submitted invoices to the</p> <p>15 plaintiffs' lawyers?</p> <p>16 A I'm sure I did.</p> <p>17 Q Do you have that documentation?</p> <p>18 A No. It's -- it's an electronic</p> <p>19 disclosure. It's not actually done on paper.</p> <p>20 Q One of the things that HudsonAlpha does</p> <p>21 is it partners with the University of Alabama in</p> <p>22 a comprehensive cancer center; correct?</p> <p>23 A No, that wouldn't be correct.</p> <p>24 HudsonAlpha is very specific --</p>	<p>1 members on both institutions.</p> <p>2 MS. BROWN:</p> <p>3 Q Fair to say, then, Doctor, you have not</p> <p>4 participated in any work with the University of</p> <p>5 Alabama's Comprehensive Cancer Center?</p> <p>6 MS. O'DELL:</p> <p>7 Object to the form.</p> <p>8 A No, that's not true.</p> <p>9 MS. BROWN:</p> <p>10 Q Have you worked with the University of</p> <p>11 Alabama's Comprehensive Cancer Center on projects</p> <p>12 involving ovarian cancer?</p> <p>13 MS. O'DELL:</p> <p>14 Objection. Asked and answered.</p> <p>15 A I would -- I would have to review the</p> <p>16 specific projects that we've -- we've done to</p> <p>17 answer that.</p> <p>18 As the codirector of a core facility</p> <p>19 for the University of Alabama Comprehensive</p> <p>20 Cancer Center, it is likely that we've worked on</p> <p>21 some projects related to ovarian cancer, but I</p> <p>22 can't specifically name them. They are -- I</p> <p>23 would -- I would characterize them as infrequent.</p> <p>24 MS. BROWN:</p>
Page 103	Page 105
<p>1 And you may be more familiar with this</p> <p>2 than I.</p> <p>3 They're very specific with their use of</p> <p>4 the word "partnership" and they're, in fact, very</p> <p>5 specific that they do not engage in a -- anything</p> <p>6 titled "a partnership." So they -- I would not</p> <p>7 characterize them as a partner of the University</p> <p>8 of Alabama Cancer Center.</p> <p>9 We certainly have -- there are faculty</p> <p>10 members at University of Alabama Birmingham who</p> <p>11 are -- have adjunct appointments at HudsonAlpha,</p> <p>12 just as I have appointments at University of</p> <p>13 Alabama Birmingham and I am a member of their</p> <p>14 cancer center.</p> <p>15 Q Are you aware of the work that</p> <p>16 HudsonAlpha does with the University of Alabama's</p> <p>17 Comprehensive Cancer Center?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form. Asked and</p> <p>20 answered.</p> <p>21 A I'm aware of some of the work, but I --</p> <p>22 certainly I -- I don't -- I don't necessarily</p> <p>23 have knowledge of the full spectrum of those</p> <p>24 projects, given that they involve many faculty</p>	<p>1 Q Have any of those projects attempted to</p> <p>2 research the potential causes of ovarian cancer?</p> <p>3 A Again, I'd have -- I'd have to review</p> <p>4 the projects. They're certainly --</p> <p>5 fundamentally, most of the questions regarding</p> <p>6 the analysis of cancer samples are routinely to</p> <p>7 investigate their cause or their treatment. So I</p> <p>8 would -- I would answer that question as highly</p> <p>9 likely.</p> <p>10 Q Would you agree the cause of ovarian</p> <p>11 cancer remains unknown today?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form.</p> <p>14 A No, I would -- I would -- I would not</p> <p>15 agree that it -- I would not agree to that</p> <p>16 general statement.</p> <p>17 MS. BROWN:</p> <p>18 Q What are the causes of ovarian cancer</p> <p>19 in your mind, Doctor?</p> <p>20 A Well, the -- the causes of -- of</p> <p>21 a -- of any number of cancers, including ovarian</p> <p>22 cancer, are probably more well understood now</p> <p>23 than ever, and their complexities I think now are</p> <p>24 just beginning to be appreciated in the sense</p>

27 (Pages 102 to 105)

Shawn Levy, Ph.D.

Page 106	Page 108
<p>1 that cancer is a disease of unregulated cell</p> <p>2 growth.</p> <p>3 Back to our earlier con- -- earlier</p> <p>4 conversation, some of the fundamental facts that</p> <p>5 we had discussed and, in fact, I think well</p> <p>6 replicated in a number of sources, as you pointed</p> <p>7 out to me, you know, illustrate that there's a</p> <p>8 milieu of genetic change leading to cellular</p> <p>9 transformation, and that cellular damage, if we</p> <p>10 consolidate that as cellular damage, then has to</p> <p>11 work in concert with a number of other events</p> <p>12 providing the right environment for a tumor to</p> <p>13 grow, such as inflammation, chronic or acute.</p> <p>14 And, so, the -- you know, the -- the -- you know,</p> <p>15 giving a singular cause would be inappropriate.</p> <p>16 But I would say the mechanistic causes</p> <p>17 of cancer are reasonably well understood, but how</p> <p>18 those apply to the wide diversity of cancer types</p> <p>19 remains an area of active investigation.</p> <p>20 I think what's interesting on cancer in</p> <p>21 general is that there's no -- really no longer a</p> <p>22 bucket diagnosis. It is -- it -- lung cancer is</p> <p>23 more complex than lung cancer and ovarian cancer,</p> <p>24 certainly with the --</p>	<p>1 Now, the -- whether that represents the</p> <p>2 complete milieu of possibilities is -- is what is</p> <p>3 currently under research.</p> <p>4 MS. BROWN:</p> <p>5 Q Were you aware that the University of</p> <p>6 Alabama Comprehensive Cancer Center is an NCI</p> <p>7 center, National Cancer Institute?</p> <p>8 A Yes. It's -- it's not only an</p> <p>9 NCI-designated center; it's an NCI-designated</p> <p>10 comprehensive cancer center, which is a slightly</p> <p>11 different classification. It's a -- there's more</p> <p>12 criteria for a cancer center to meet to become</p> <p>13 comprehensive.</p> <p>14 Q What does it mean to be an NCI center,</p> <p>15 to you, if you know?</p> <p>16 A Stated very simply, it means you have</p> <p>17 a -- your cancer center is funded by a support</p> <p>18 grant directly from the National Cancer Institute</p> <p>19 to provide -- that supports not only patient care</p> <p>20 but also supports basic research, epidemiology</p> <p>21 and -- and health outcomes research in cancer.</p> <p>22 So, in a nutshell, it is a fairly</p> <p>23 comprehensive grant that supports a wide variety</p> <p>24 of work within a cancer center that extends</p>
Page 107	Page 109
<p>1 As I'm sure you're well aware, with the</p> <p>2 molecular subtypes and other things, it's a</p> <p>3 complicated disease as well.</p> <p>4 So to summarize that is -- to summarize</p> <p>5 all of that complexity by saying that the cause</p> <p>6 is known or unknown I think would vastly</p> <p>7 underestimate the -- our current state of the art</p> <p>8 or knowledge of how complex cancer is as a</p> <p>9 condition.</p> <p>10 Q Sure.</p> <p>11 Scientists, researchers, public health</p> <p>12 authorities continue to investigate the mechanism</p> <p>13 by which ovarian cancer is caused. Correct?</p> <p>14 A That's correct.</p> <p>15 Q We do not, sitting here today in 2019,</p> <p>16 have a complete understanding of the etiology of</p> <p>17 ovarian cancer. Correct?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A I would say we have substantial</p> <p>21 knowledge of factors and exposures that either</p> <p>22 predispose or directly cause cancer in a large</p> <p>23 number of -- large number of cancer areas,</p> <p>24 including ovarian cancer.</p>	<p>1 beyond basic -- basic care.</p> <p>2 Q The National Cancer Institute has</p> <p>3 funded a number of projects that the scientists</p> <p>4 at HudsonAlpha are working on. Is that fair?</p> <p>5 A I'd have to certainly review the grant</p> <p>6 portfolio. But I'm certain that, since I myself</p> <p>7 have funding from that cancer center, yes, the</p> <p>8 NCI does fund some -- some number of</p> <p>9 investigators at HudsonAlpha.</p> <p>10 Q And you consider the NCI to be a</p> <p>11 reputable public health authority; correct?</p> <p>12 A No, not necessarily. The NCI is really</p> <p>13 not a public health authority. The N -- the NCI</p> <p>14 is a -- is a scientific administration center</p> <p>15 within the National Institutes of Health.</p> <p>16 Now, I'm speaking of their extramural</p> <p>17 programs. The NCI also have intramural programs,</p> <p>18 where they have their own researchers and their</p> <p>19 own projects. I'm less familiar with those</p> <p>20 activities.</p> <p>21 But together, I would state that the</p> <p>22 NCI is a -- I don't have -- I guess I have not</p> <p>23 had any experience with the NCI that would lead</p> <p>24 me to say that they are an authoritative public</p>

28 (Pages 106 to 109)

Shawn Levy, Ph.D.

Page 110	Page 112
<p>1 health authority.</p> <p>2 Q Before forming your opinions in this</p> <p>3 case, Dr. Levy, did you look to see what the NCI</p> <p>4 states about whether talcum powder causes ovarian</p> <p>5 cancer?</p> <p>6 A I believe I did see, from a number of</p> <p>7 statements, certainly potentially from the NCI,</p> <p>8 regarding the complete opinion and -- and</p> <p>9 knowledge base for the role of talcum powder in</p> <p>10 ovarian cancer.</p> <p>11 Q Do you recall that the NCI has</p> <p>12 concluded that there's inadequate evidence that</p> <p>13 talcum powder increases the risk of ovarian</p> <p>14 cancer?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 A Which -- what specifically are you</p> <p>18 referring to? I -- I wouldn't be able to answer</p> <p>19 that accurately without knowing which specific</p> <p>20 report or statement that you're referring to.</p> <p>21 MS. BROWN:</p> <p>22 Q I'm wondering if, sitting here today,</p> <p>23 you recall looking at information about the</p> <p>24 classification of risk factors for ovarian cancer</p>	<p>1 you are prepared to offer the opinion that talcum</p> <p>2 powder causes ovarian cancer.</p> <p>3 A I don't -- I don't think we have the</p> <p>4 complete information for a sing- -- you know, to</p> <p>5 have the opinion of a singular cause. But, at</p> <p>6 the same time, my opinions are that, as stated in</p> <p>7 the report, there's a clear and well-evidenced</p> <p>8 biologically plausible role for talcum powder</p> <p>9 leading to ovarian cancer.</p> <p>10 Q On page 2 of your report, the second</p> <p>11 full paragraph that begins "My report</p> <p>12 consists" --</p> <p>13 You with me?</p> <p>14 A Yes.</p> <p>15 Q -- you state -- you reference your</p> <p>16 conclusions regarding this cause-and-effect</p> <p>17 relationship.</p> <p>18 Do you see that?</p> <p>19 A I do.</p> <p>20 Q Do you mean by that that you have an</p> <p>21 opinion that talcum powder causes the effect of</p> <p>22 ovarian cancer?</p> <p>23 A No. That -- that wasn't the meaning of</p> <p>24 that statement of cause and effect. It was -- it</p>
Page 111	Page 113
<p>1 as done by the NCI.</p> <p>2 A I don't recall that specifically. I</p> <p>3 don't also recall seeing any statements from the</p> <p>4 NCI regarding safety of any product.</p> <p>5 Q In forming your opinions in this case,</p> <p>6 Dr. Levy, did you consider the conclusions of</p> <p>7 public health authorities like the FDA, the NCI,</p> <p>8 NIH as it relates to talcum powder in ovarian</p> <p>9 cancer?</p> <p>10 A So I certainly considered information</p> <p>11 from each of those entities. But I would make a</p> <p>12 statement I don't -- I don't recall from any of</p> <p>13 those entities seeing a single conclusion.</p> <p>14 Q Is it your opinion, Dr. Levy, that</p> <p>15 talcum powder causes ovarian cancer?</p> <p>16 A I wasn't asked to provide an opinion if</p> <p>17 talcum powder causes cancer. I was -- I was</p> <p>18 asked to develop an opinion as to the biological</p> <p>19 plausibility of -- of talcum powder leading</p> <p>20 to -- leading to change.</p> <p>21 Now, that's what I was asked from the</p> <p>22 attorneys. If you're asking -- are you asking me</p> <p>23 what my opinion is --</p> <p>24 Q Well, I want to know if, in this case,</p>	<p>1 was a -- more of a general statement of a cause</p> <p>2 being exposure to talc and effect being that</p> <p>3 biologically plausible mechanism.</p> <p>4 Q You mentioned a moment ago that you</p> <p>5 don't think we have the complete info on a</p> <p>6 singular cause of ovarian cancer. Is that right?</p> <p>7 MS. O'DELL:</p> <p>8 Objection to form.</p> <p>9 A Sorry. Let me read your question</p> <p>10 again.</p> <p>11 I have -- I have not seen any evidence</p> <p>12 that suggests that there is a singular cause of</p> <p>13 ovarian cancer.</p> <p>14 MS. BROWN:</p> <p>15 Q You have not seen sufficient evidence</p> <p>16 to suggest that talcum powder could be one of the</p> <p>17 causes of ovarian cancer; correct?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A I would disagree. As -- as stated,</p> <p>21 the -- I have not seen evidence that there's a</p> <p>22 singular cause of ovarian cancer. I think there</p> <p>23 is ample evidence that there are a multitude of</p> <p>24 mechanisms that you can get cellular damage and</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 114</p> <p>1 cellular change within the ovary which then leads</p> <p>2 to malignant transformation, and that, as stated</p> <p>3 in the report, there's a biologically plausible</p> <p>4 mechanism that exposure to talcum powder and its</p> <p>5 constituents can create those necessary changes.</p> <p>6 MS. BROWN:</p> <p>7 Q Do you believe, Doctor, there's</p> <p>8 sufficient evidence that talcum powder, through</p> <p>9 chronic inflammation, causes ovarian cancer in</p> <p>10 some individuals?</p> <p>11 A No. That -- that was not my -- not my</p> <p>12 opinion or statement. And I would say</p> <p>13 specifically chronic inflammation is, again,</p> <p>14 narrowing the focus in an inappropriate way, and</p> <p>15 the evidence doesn't illustrate that chronic</p> <p>16 inflammation is a singular sufficient detail or,</p> <p>17 I should say, effect to result in ovarian cancer.</p> <p>18 It's certainly a factor, as -- as well described</p> <p>19 in the -- in the literature.</p> <p>20 And -- and, again, I would defer to</p> <p>21 other expert reports that have similar opinions</p> <p>22 regarding inflammation, chronic inflammation</p> <p>23 being one of them.</p> <p>24 And it may be important to provide an</p>	<p style="text-align: right;">Page 116</p> <p>1 of observations and studies that</p> <p>2 have -- certainly exist. And, again, their</p> <p>3 review and -- and content is what went to the</p> <p>4 opinions in my report.</p> <p>5 Q And most of the studies that you cite,</p> <p>6 Dr. Levy, talking about chronic inflammation</p> <p>7 refer to chronic inflammation as a hypothesis of</p> <p>8 one of the ways cancer might form in the ovary.</p> <p>9 Correct?</p> <p>10 MS. O'DELL:</p> <p>11 Object to the form.</p> <p>12 A Let me -- sorry. Let me read your</p> <p>13 question.</p> <p>14 No. I would disagree. At least,</p> <p>15 certainly not most of the studies that I cite.</p> <p>16 MS. BROWN:</p> <p>17 Q Do you believe chronic inflammation is</p> <p>18 an established mechanism of ovarian cancer?</p> <p>19 A Yes, in the sense that chronic</p> <p>20 inflammation is a well-established mechanism of</p> <p>21 cancer in general, including ovarian cancer.</p> <p>22 This is first observed in the 1800s and has since</p> <p>23 been -- become well-established in the -- in the</p> <p>24 cancer field that inflammation plays a</p>
<p style="text-align: right;">Page 115</p> <p>1 important distinction that cellular damage or</p> <p>2 what we can refer to as acute inflammation can</p> <p>3 cause -- certainly has been shown and is</p> <p>4 well-evidenced that it causes -- can lead to</p> <p>5 molecular changes that can lead to cancer.</p> <p>6 Chronic inflammation is a slightly --</p> <p>7 is in a slightly different biological perspective</p> <p>8 in that it provides the correct environment for</p> <p>9 those cancerous changes to take hold and allow</p> <p>10 malignant transformation, as I mentioned.</p> <p>11 So I -- I do view them as working in</p> <p>12 concert but not necessarily independent. So when</p> <p>13 you ask a question that specifically narrows it</p> <p>14 to chronic inflammation or even acute</p> <p>15 inflammation in a singular fashion, you know, my</p> <p>16 answers will largely be the same, that that's, in</p> <p>17 and of itself, is too limited to describe as a</p> <p>18 specific cause, singular or otherwise, of ovarian</p> <p>19 cancer or of cancer in general.</p> <p>20 Q You'd agree that the research regarding</p> <p>21 whether chronic inflammation can cause ovarian</p> <p>22 cancer is ongoing?</p> <p>23 A Yes, I would agree it is -- it is</p> <p>24 ongoing research. But there are a large number</p>	<p style="text-align: right;">Page 117</p> <p>1 significant role in both the initiation as well</p> <p>2 as progression of cancer.</p> <p>3 Q What methodology did you employ for</p> <p>4 coming to the opinion that chronic inflammation</p> <p>5 is a well-established cause of ovarian cancer?</p> <p>6 A Just general mechanism in terms of</p> <p>7 evaluating biological plausibility.</p> <p>8 Q I understand, Dr. Levy, you have a</p> <p>9 general opinion that chronic inflammation can</p> <p>10 lead to some cancer. Is that right?</p> <p>11 MS. O'DELL:</p> <p>12 Objection to form. Misstates his</p> <p>13 testimony.</p> <p>14 A I -- I have an opinion regarding the</p> <p>15 role and importance of inflammation in the</p> <p>16 initiation and progression of cancer.</p> <p>17 MS. BROWN:</p> <p>18 Q And, as it relates to ovarian cancer,</p> <p>19 what methodology did you employ to arrive at your</p> <p>20 conclusion that chronic inflammation is an</p> <p>21 established cause of ovarian cancer?</p> <p>22 A I -- I did not arrive at that specific</p> <p>23 conclusion, nor was I asked to.</p> <p>24 Q You do not believe that chronic</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 118</p> <p>1 inflammation has been established as a cause of</p> <p>2 ovarian cancer; correct?</p> <p>3 MS. O'DELL:</p> <p>4 Object to the form.</p> <p>5 A No, that -- that's not what I said.</p> <p>6 MS. BROWN:</p> <p>7 Q Explain it to me.</p> <p>8 A I've stated that chronic inflammation</p> <p>9 or inflammation in general, including chronic and</p> <p>10 acute infor -- inflammation, is a component and a</p> <p>11 necessary component for the initiation and</p> <p>12 progression of -- of cancer as we understand it</p> <p>13 today. And, in that, cancer, certainly ovarian</p> <p>14 cancer as well as a variety of other cancer</p> <p>15 types, is included.</p> <p>16 Q What methodology did you employ to</p> <p>17 arrive at the conclusion that ovarian cancer is</p> <p>18 one of the cancers that can be caused by chronic</p> <p>19 inflammation?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form. Misstates his</p> <p>22 testimony.</p> <p>23 A Yeah. Again, we're not -- I'm not</p> <p>24 making a specific causal opinion with respect to</p>	<p style="text-align: right;">Page 120</p> <p>1 from animal models to in vitro studies, in vivo</p> <p>2 studies, cohort studies, case-control studies.</p> <p>3 There was quite a broad spectrum of information</p> <p>4 across a large number of years.</p> <p>5 Q Do you believe you reviewed the</p> <p>6 totality of the epidemiology on talcum powder use</p> <p>7 and ovarian cancer?</p> <p>8 MS. O'DELL:</p> <p>9 Object to the form.</p> <p>10 A I -- I reviewed the available studies</p> <p>11 that appeared to be relevant for the -- for the</p> <p>12 opinions that are expressed in my report.</p> <p>13 MS. BROWN:</p> <p>14 Q And when you say "available," what do</p> <p>15 you mean?</p> <p>16 A Meaning that I could -- I could</p> <p>17 discover in the scientific literature.</p> <p>18 Q Did you conduct your own literature</p> <p>19 searches in connection with your work in this</p> <p>20 case?</p> <p>21 A I did.</p> <p>22 Q How did you go about finding the</p> <p>23 totality of the evidence relating to whether</p> <p>24 talcum powder causes ovarian cancer?</p>
<p style="text-align: right;">Page 119</p> <p>1 any -- whether -- whether inflammation, talcum</p> <p>2 powder use or other exposures. I -- my -- my</p> <p>3 opinion in the report is -- is -- was not asked</p> <p>4 to be a causal opinion.</p> <p>5 MS. BROWN:</p> <p>6 Q You reference on page 2 of your report</p> <p>7 that your opinions are based on assessing and</p> <p>8 weighing the totality of the evidence, including</p> <p>9 relevant literature and available documentation</p> <p>10 and your experience as a geneticist and</p> <p>11 scientific researcher. Do you see that?</p> <p>12 A Yes.</p> <p>13 Q What do you mean by "the totality of</p> <p>14 the evidence"?</p> <p>15 A All of the evidence available at the</p> <p>16 time that I was researching this report.</p> <p>17 Q All of the evidence concerning what?</p> <p>18 A Concerning a variety of subjects</p> <p>19 surrounding ovarian cancer, talcum powder use,</p> <p>20 and then inflammation and related subjects as my</p> <p>21 literature review and review of available</p> <p>22 information progressed.</p> <p>23 So there was a, I guess, a large number</p> <p>24 of tangential directions that -- that I examined,</p>	<p style="text-align: right;">Page 121</p> <p>1 A So the -- my methodology for the</p> <p>2 literature review in establishing my opinion</p> <p>3 regarding the biological plausibility of talcum</p> <p>4 powder exposure inflammation and its potential</p> <p>5 role in ovarian cancer was based on, you know, my</p> <p>6 activities and many other literature searches, so</p> <p>7 using a variety of computational tools and -- and</p> <p>8 web-based resources, from journals to, I would</p> <p>9 say, primarily PubMed being a resource, but also</p> <p>10 ISI, Web of Science, Google Scholar and a variety</p> <p>11 of -- bioRxiv and I'm sure a number of other</p> <p>12 sources. But those were probably the more</p> <p>13 primary resources for establishing what</p> <p>14 literature was available.</p> <p>15 Q Did you ask the plaintiffs' lawyers for</p> <p>16 any scientific literature that you used in</p> <p>17 forming your opinions in this case?</p> <p>18 A What do you mean by "ask"? There</p> <p>19 is -- as far as did I ask for their similar</p> <p>20 process, no.</p> <p>21 There were some papers that I had</p> <p>22 identified but was not able to access the full</p> <p>23 content via the libraries that I have access to.</p> <p>24 So in some of those cases, specific references</p>

31 (Pages 118 to 121)

Shawn Levy, Ph.D.

Page 122	Page 124
<p>1 that I provided, those full -- that full content</p> <p>2 was provided by the plaintiffs' lawyer to allow</p> <p>3 me to review it.</p> <p>4 Q Did the plaintiffs' lawyers give you a</p> <p>5 set of epidemiology on which you're relying on to</p> <p>6 form your opinion?</p> <p>7 A No, they did not.</p> <p>8 Q If I look at your report, I see a</p> <p>9 reference list and then a separate Exhibit B. Is</p> <p>10 that right?</p> <p>11 A Yes.</p> <p>12 Q So, for example, on page 18 of your</p> <p>13 report, you have a list of literature cited.</p> <p>14 Correct?</p> <p>15 A Yes.</p> <p>16 Let me make sure I have the page</p> <p>17 correct.</p> <p>18 Yes, beginning on page 18.</p> <p>19 Q Is everything that appears in the</p> <p>20 literature-cited list something that you found on</p> <p>21 your own, Dr. Levy?</p> <p>22 A I would have to review the -- the list.</p> <p>23 But there are certainly --</p> <p>24 Let me --</p>	<p>1 relying on information in that article to form</p> <p>2 your opinions in this case?</p> <p>3 A No. I'm not relying on any singular</p> <p>4 article or source to form my opinion on the case.</p> <p>5 Q Are you relying in part on the</p> <p>6 information contained in the Blount article?</p> <p>7 A Since I include it in the cited</p> <p>8 literature, certainly in some -- in some part.</p> <p>9 Q What information are you relying on in</p> <p>10 the Blount article?</p> <p>11 A I would have to review the article to</p> <p>12 remind myself where the --</p> <p>13 Q Take a look at it. We'll pull it right</p> <p>14 now.</p> <p>15 What about Paoletti on page 22? Was</p> <p>16 that something you found on your own or did the</p> <p>17 lawyers give you that?</p> <p>18 A So Paoletti --</p> <p>19 Q Uh-huh.</p> <p>20 A Page 22?</p> <p>21 Q Uh-huh.</p> <p>22 A Actually, the Paoletti one is familiar.</p> <p>23 That's an interesting one because it's in</p> <p>24 Italian.</p>
Page 123	Page 125
<p>1 I believe the Saed abstracts, as an</p> <p>2 example --</p> <p>3 Let me see if there are --</p> <p>4 No. I -- I believe, in the literature</p> <p>5 cited, there are certainly some number of</p> <p>6 examples of information that was provided during</p> <p>7 the course of the development of my report from</p> <p>8 the plaintiffs' attorneys in terms of literature</p> <p>9 for my consideration, but that in no case -- in</p> <p>10 every case it was provided as a -- as</p> <p>11 information.</p> <p>12 The vast majority or nearly the</p> <p>13 totality of this was information that I had --</p> <p>14 that I indeed discovered myself and shared with</p> <p>15 the -- the attorneys, but certainly not complete.</p> <p>16 Q On page 18 you cite an article by</p> <p>17 Blount.</p> <p>18 Do you see that?</p> <p>19 A Yes.</p> <p>20 Q Was that given to you by the</p> <p>21 plaintiffs' lawyers?</p> <p>22 A I'd have to look at my records. I</p> <p>23 don't recall.</p> <p>24 Q Off of the top of your head, are you</p>	<p>1 Q Are you relying on the information in</p> <p>2 the Paoletti article to form your opinions in the</p> <p>3 case?</p> <p>4 A Again, the -- I wasn't relying on any</p> <p>5 singular article but instead tried to present and</p> <p>6 provide reference to as comprehensive a</p> <p>7 collection of relevant literature in this -- in</p> <p>8 this space as possible, of which Paoletti,</p> <p>9 although being in Italian, there were some --</p> <p>10 enough translated aspects of that that it was</p> <p>11 worthy to include in the -- in that cited</p> <p>12 literature as being relevant to the -- to</p> <p>13 those -- to those opinions.</p> <p>14 Q Just to make sure we get on the same</p> <p>15 page here, Dr. Levy, when I ask are you relying</p> <p>16 on something, I don't mean by that question to</p> <p>17 suggest it's the only thing you're relying on.</p> <p>18 And I'll try to say "in part" to make it easy for</p> <p>19 us. Okay?</p> <p>20 A Right. Just want to be -- make sure</p> <p>21 we're clear.</p> <p>22 Q Absolutely. So do I.</p> <p>23 And I want to know are you relying in</p> <p>24 part on anything in the Paoletti article to form</p>

Shawn Levy, Ph.D.

Page 126	Page 128
<p>1 your opinions in this case?</p> <p>2 A I would say in -- in part. As far as</p> <p>3 my opinions regarding the biologically plausible</p> <p>4 mechanism that was presented, no, it does not</p> <p>5 rely on that specific conclusions of that paper</p> <p>6 but, rather, that paper was included because of</p> <p>7 its results regarding asbestos contamination in</p> <p>8 industrial talc, which only support -- add</p> <p>9 support to the mechanism that I presented in the</p> <p>10 report.</p> <p>11 Q Is your opinion in this case, Doctor,</p> <p>12 based on an assumption that baby powder contains</p> <p>13 asbestos?</p> <p>14 A No, it is not.</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 MS. BROWN:</p> <p>18 Q Is your opinion in this case based on</p> <p>19 an assumption that baby powder contains</p> <p>20 fragrances?</p> <p>21 MS. O'DELL:</p> <p>22 Objection to form.</p> <p>23 A My -- my opinion considers the totality</p> <p>24 of the constituent components of baby powder,</p>	<p>1 presented.</p> <p>2 MS. BROWN:</p> <p>3 Q Do you believe that baby talc alone can</p> <p>4 cause inflammation that may lead to ovarian</p> <p>5 cancer?</p> <p>6 A Based on my review of the literature,</p> <p>7 there are a number of studies, both of those</p> <p>8 involving human studies in terms of case</p> <p>9 controls, as well as a number of animal studies</p> <p>10 and then, more specifically, in vitro studies</p> <p>11 that look at talcum powder and its ability to</p> <p>12 produce clear markers of inflammation.</p> <p>13 I am -- the -- I am not aware of any</p> <p>14 specific testing that looked at platy talc</p> <p>15 individually as a singular component without</p> <p>16 the -- or out of the context of the products we</p> <p>17 were just describing in a similar analysis. So I</p> <p>18 don't -- I don't know that answer.</p> <p>19 Q Is it your opinion that</p> <p>20 Johnson & Johnson baby powder products are</p> <p>21 contaminated with asbestos?</p> <p>22 MS. O'DELL:</p> <p>23 Object to the form. Asked and</p> <p>24 answered.</p>
Page 127	Page 129
<p>1 Shower to Shower, you know, under -- either, as</p> <p>2 we've been referring to it simply as talc or</p> <p>3 talcum powder or by trade names such as</p> <p>4 Johnson & Johnson or Shower to Shower, so the --</p> <p>5 my opinions, as stated in the report, being</p> <p>6 reasonably -- or trying to be reasonably</p> <p>7 comprehensive. Therefore, it's not, you know,</p> <p>8 limited to any -- any singular component, whether</p> <p>9 it be majority or minority, in the -- in the</p> <p>10 talcum powder products, as I just stated.</p> <p>11 MS. BROWN:</p> <p>12 Q Is your opinion in this case based on</p> <p>13 an assumption that Johnson & Johnson baby powder</p> <p>14 products contain heavy metals?</p> <p>15 MS. O'DELL:</p> <p>16 Objection to form.</p> <p>17 A Again, similar to the earlier</p> <p>18 statement, the opinion is not subject to</p> <p>19 any -- any singular component. I think the</p> <p>20 information regarding the -- in deferring to some</p> <p>21 of the other experts regarding the knowledge of</p> <p>22 constituent components, whether they be heavy</p> <p>23 metals or asbestos, only helps to support the</p> <p>24 biological plausibility of the mechanism I</p>	<p>1 A I -- I -- I have -- I have been</p> <p>2 provided expert report, and some of those are</p> <p>3 referenced in the -- in the report, as we were</p> <p>4 describing, that describe testing of a number</p> <p>5 of -- number of samples,</p> <p>6 included -- Johnson & Johnson included in that,</p> <p>7 that showed how they -- that the results of those</p> <p>8 reports showed contamination by asbestos or --</p> <p>9 or -- or asbestos-like fiber. So, therefore,</p> <p>10 I've been presented with that evidence.</p> <p>11 MS. BROWN:</p> <p>12 Q Have you relied on that evidence in</p> <p>13 forming your opinions in this case?</p> <p>14 A Again, no, not -- not as a singular</p> <p>15 evidence. So, as we just discussed a moment ago,</p> <p>16 that is a component piece of evidence that</p> <p>17 leads -- and is supportive of the biologically</p> <p>18 plausible mechanism described in the report.</p> <p>19 You know, certainly, it is inarguable</p> <p>20 that asbestos and asbestos-like fibers cause</p> <p>21 inflammation. There's also ample evidence of the</p> <p>22 inflammatory effects of talc. And -- and talc</p> <p>23 pleurodesis, for example, is -- is designed to</p> <p>24 produce inflammatory response as a treatment.</p>

Shawn Levy, Ph.D.

Page 130	Page 132
<p>1 So I think, again, similar to the</p> <p>2 relationship of asbestos and inflammation, it's a</p> <p>3 well-established scientific fact that talc has an</p> <p>4 inflammatory role now. Or I should say as of</p> <p>5 today.</p> <p>6 Q Have you attempted to quantify, based</p> <p>7 on the reports of Dr. Longo that you reviewed,</p> <p>8 how much asbestos contamination is in</p> <p>9 Johnson & Johnson baby powder products?</p> <p>10 MS. O'DELL:</p> <p>11 Objection. Vague as to form.</p> <p>12 A I --</p> <p>13 MS. O'DELL:</p> <p>14 As to the volume and time contained,</p> <p>15 et cetera.</p> <p>16 A My -- my answer is simply that I wasn't</p> <p>17 asked to quantify that as part of my report.</p> <p>18 MS. BROWN:</p> <p>19 Q Whether there is asbestos in Johnson &</p> <p>20 Johnson baby powder products or not does not</p> <p>21 impact your opinions in this case; is that right?</p> <p>22 MS. O'DELL:</p> <p>23 Object to the form.</p> <p>24 A The opinions regarding the biological</p>	<p>1 in any of the above-referenced studies.</p> <p>2 MS. O'DELL:</p> <p>3 Objection. Misstates his testimony.</p> <p>4 A So reading -- reading back my</p> <p>5 testimony --</p> <p>6 MS. BROWN:</p> <p>7 Q So, Doctor, I see that you're looking</p> <p>8 at the realtime?</p> <p>9 A Yes.</p> <p>10 Q To get clarification on the question?</p> <p>11 A No. To -- to remem- -- to -- you asked</p> <p>12 me a question about my statement.</p> <p>13 Q Correct.</p> <p>14 A And I was reviewing specifically what I</p> <p>15 had stated so I could answer your question</p> <p>16 accurately.</p> <p>17 Q Terrific. So I want to know what you</p> <p>18 were talking about when you said you were unable</p> <p>19 to discover the contamination rate.</p> <p>20 A To clarify, I was not asked to estimate</p> <p>21 or determine the contamination rate, and my</p> <p>22 statement regarding that was in reference to the</p> <p>23 material I reviewed and the literature that is</p> <p>24 referenced in my report. I don't recall in any</p>
Page 131	Page 133
<p>1 plausibility described in my report and its</p> <p>2 relationship to asbestos are somewhat separate,</p> <p>3 meaning that I have -- I was not able to discover</p> <p>4 what the contamination rate or content of</p> <p>5 asbestos was in any of the referenced studies</p> <p>6 through the course of my report, so, therefore, I</p> <p>7 can't comment on the likelihood or -- of -- of</p> <p>8 how many or any -- or any or all of those samples</p> <p>9 contain asbestos.</p> <p>10 MS. BROWN:</p> <p>11 Q And sounds like you did some work</p> <p>12 attempting to see if you could calculate a</p> <p>13 contamination rate. Is that what you were</p> <p>14 describing?</p> <p>15 MS. O'DELL:</p> <p>16 Object -- object to the form.</p> <p>17 Misstates his testimony.</p> <p>18 A No. No, not at all. I stated that I</p> <p>19 didn't have information available to assess</p> <p>20 either -- either way.</p> <p>21 MS. BROWN:</p> <p>22 Q Tell me what you meant when you</p> <p>23 testified that you were not able to discover what</p> <p>24 the contamination rate or content of asbestos was</p>	<p>1 of those studies observing a specific statement</p> <p>2 of amount of asbestos in the talcum powder</p> <p>3 products that were under study. So, therefore, I</p> <p>4 am not able to form an opinion surrounding that</p> <p>5 contamination rate.</p> <p>6 Q Would the same be true, Doctor, for</p> <p>7 heavy metals?</p> <p>8 A Yes, that's correct.</p> <p>9 Q And when I say the same would be true,</p> <p>10 that means you were not able to calculate a rate</p> <p>11 of heavy metal contamination of any of the talcum</p> <p>12 powder products in the studies you reviewed?</p> <p>13 MS. O'DELL:</p> <p>14 Objection. Vague.</p> <p>15 A I was not asked to.</p> <p>16 MS. BROWN:</p> <p>17 Q Did you attempt to quantify the amount</p> <p>18 of heavy metals?</p> <p>19 MS. O'DELL:</p> <p>20 Objection.</p> <p>21 A I certainly reviewed the literature to</p> <p>22 understand what information was available</p> <p>23 regarding the products that may have been used</p> <p>24 and what testing may have been done on</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 134</p> <p>1 those -- on those products. 2 MS. BROWN: 3 Q And, as it relates to fragrances, have 4 you calculated the amount of fragrances that are 5 present in Johnson & Johnson's baby powder 6 products? 7 MS. O'DELL: 8 Objection to form. 9 A I -- I wasn't asked to -- to make those 10 calculations. And I would defer to other expert 11 reports that I had an opportunity to review 12 recently that did perform those calculations. 13 MS. BROWN: 14 Q Your opinions in this case are not 15 dependent on whether or not -- 16 A I think that was -- 17 Q -- there are fragrances in 18 Johnson & Johnson's baby powder; correct? 19 MS. O'DELL: 20 Objection. 21 A Sorry. Let me read that. 22 Sorry. Could you rephrase your 23 question? The question that appears on the 24 monitor is that there are fragrances in</p>	<p style="text-align: right;">Page 136</p> <p>1 fragrances as well as asbestos, I would say my 2 opinion now is that that information continues to 3 support the biologically plausible mechanism 4 presented in my report. 5 MS. BROWN: 6 Q Your opinion that chronic inflammation 7 is a biologically plausible mechanism by which 8 talcum powder could cause ovarian cancer is not 9 dependent on heavy metals being present in talcum 10 powder; correct? 11 MS. O'DELL: 12 Object to the form. Asked and 13 answered. 14 A My -- my opinions are not based on -- 15 on any singular component or constituent because 16 the -- the available information did not 17 scientifically test any singular components 18 or -- or allow -- 19 I'm not aware of any studies that 20 examine the inflammatory or other effects of 21 talcum powder that contained heavy metals versus 22 did not. 23 MS. BROWN: 24 Q So, for purposes of your opinions in</p>
<p style="text-align: right;">Page 135</p> <p>1 Johnson & Johnson baby powder, question mark. 2 MS. BROWN: 3 Q That's why it's tricky when you read 4 the realtime. Just listen to my question. It'll 5 be more helpful. 6 Your opinion in this case is not 7 dependent on whether or not there are fragrances 8 in Johnson & Johnson baby powder. Correct? 9 MS. O'DELL: 10 Excuse me. Objection to form. 11 You may refer to realtime any time you 12 want to, Doctor. 13 But I object to the form of the 14 question. 15 A So my -- my -- I was -- what was 16 requested of me, again, stating for clarity, was 17 to describe a biologically plausible mechanism 18 for talc and all of its constituent components 19 having a role in inflammation and progression to 20 ovarian cancer based on -- on the information at 21 hand. 22 Certainly the fact, as we've been 23 provided later, the ex- -- the recent review of 24 some other expert reports regarding the</p>	<p style="text-align: right;">Page 137</p> <p>1 this case, for your piece of the puzzle, so to 2 speak, it is not important to you whether or not 3 there are heavy metals in baby powder; correct? 4 MS. O'DELL: 5 Objection to form. Asked and answered. 6 A No, that's not correct. I would say 7 the presence of all of the constituent components 8 is very important for -- from the -- from the 9 perspective of that biologically plausible 10 mechanism, and that includes the type of talc, 11 the structure of the talc, you know, its -- any 12 potential contaminants that are there, as well as 13 the complete spectrum of other constituent 14 components, fragrances, heavy metals. 15 And, of course, fragrances have their 16 own milieu of constituent components that, again, 17 I was not asked to comment on or describe in 18 detail but certainly are part of the overall 19 studies. 20 MS. BROWN: 21 Q You have a conclusion in your report on 22 page 17, Doctor, conclusion number 2, that talcum 23 powder products cause chronic inflammation. 24 Do you see that?</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 138</p> <p>1 A Yes.</p> <p>2 And I would -- and then my conclu- --</p> <p>3 Q Hold on. No question yet.</p> <p>4 A Okay.</p> <p>5 Q And what I want to know, Doctor, is how</p> <p>6 do you define the talcum powder products that</p> <p>7 you've listed here on page 17 of your report?</p> <p>8 A Primarily the products that are -- when</p> <p>9 I consider the totality of everything that I've</p> <p>10 been examining, the talcum powder products,</p> <p>11 including Johnson & Johnson and Shower to Shower</p> <p>12 as, you know, I refer to those consumer products</p> <p>13 under the term "talcum powder."</p> <p>14 Q What about other consumer talcum powder</p> <p>15 products? Are they included in your conclusions</p> <p>16 here on page 17?</p> <p>17 MS. O'DELL:</p> <p>18 Object to the form.</p> <p>19 A So my -- my conclusions are based on</p> <p>20 the -- on the literature review. And, similar to</p> <p>21 our discussions regarding contaminants and the</p> <p>22 ability to quantitate those, many of the studies</p> <p>23 did not specifically delineate which product or</p> <p>24 the timing of that product.</p>	<p style="text-align: right;">Page 140</p> <p>1 don't know if any of the studies used -- used</p> <p>2 that. I'd have to, again, would have to review</p> <p>3 some of that information to determine if there</p> <p>4 was a -- if that was a variable in any of the</p> <p>5 given studies that are the basis of the report.</p> <p>6 Q What methodology did you employ here in</p> <p>7 coming to your conclusion that chronic</p> <p>8 inflammation is caused by talcum powder products?</p> <p>9 MS. O'DELL:</p> <p>10 Objection. Asked and answered.</p> <p>11 A Yeah. Again, to restate, similar to</p> <p>12 the earlier questions, the -- my methodology was</p> <p>13 based on standard methodology for establishing</p> <p>14 biological plausibility, which is a, in a</p> <p>15 summary, a review of the totality of the evidence</p> <p>16 and then a summary of that to establish if, based</p> <p>17 on established or -- or known or factual</p> <p>18 principles, is there a -- can -- can a mechanism</p> <p>19 described go from cause to effect in a -- again,</p> <p>20 in an evidence-supported biologically plausible</p> <p>21 manner.</p> <p>22 There's a few references I can provide</p> <p>23 you that describe that method in a published</p> <p>24 manner, if that's helpful.</p>
<p style="text-align: right;">Page 139</p> <p>1 In contrast, some of the more recent</p> <p>2 information available specific to the</p> <p>3 constituents did meet that definition, so I would</p> <p>4 say these conclusions apply to both the specific</p> <p>5 products that I mentioned, Johnson & Johnson and</p> <p>6 Shower to Shower, as well as potentially other</p> <p>7 products. But quant- -- quantifying which study,</p> <p>8 I would have to go through study by study to</p> <p>9 answer any questions about which specific may be</p> <p>10 included.</p> <p>11 MS. BROWN:</p> <p>12 Q Do you include talc-containing</p> <p>13 deodorizing sprays in your definition of a talcum</p> <p>14 powder product?</p> <p>15 A None of the literature that -- that I</p> <p>16 reviewed or can recall was limited to those</p> <p>17 deodorant sprays in terms of a -- as a study</p> <p>18 variable that I can -- that I can think of.</p> <p>19 Q I'm not sure what you mean by that.</p> <p>20 A So the -- the basis of this report was</p> <p>21 on the talcum powder products, and I don't recall</p> <p>22 any of the studies that delineated talcum powder</p> <p>23 as a powder versus a talc-containing deodorant</p> <p>24 spray as a -- as a variable in the study. So I</p>	<p style="text-align: right;">Page 141</p> <p>1 MS. BROWN:</p> <p>2 Q That would be helpful.</p> <p>3 A They are -- these are our --</p> <p>4 MS. O'DELL:</p> <p>5 These are mine.</p> <p>6 THE WITNESS:</p> <p>7 Yeah.</p> <p>8 There's a -- I can get them --</p> <p>9 MS. BROWN:</p> <p>10 Q Are the published methods referenced in</p> <p>11 your report, Doctor?</p> <p>12 A No, actually, those are not.</p> <p>13 Q Okay. How would you go about finding</p> <p>14 the published methods that contain a description</p> <p>15 of the methodology you employed in this case?</p> <p>16 A No. It's that I was just saying that</p> <p>17 there's a published -- peer-reviewed published</p> <p>18 article that is the same as the method I used, if</p> <p>19 you -- if you wanted to review that. I didn't</p> <p>20 reference this specific paper in the report.</p> <p>21 Q Okay. And you have a -- do you have a</p> <p>22 copy of that in front of you right now, Doctor?</p> <p>23 A I do.</p> <p>24 Q Okay. So let's mark that as Exhibit</p>

36 (Pages 138 to 141)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 142</p> <p>1 14. 2 (DEPOSITION EXHIBIT NUMBER 14 3 WAS MARKED FOR IDENTIFICATION.) 4 MS. BROWN: 5 Q The title of the document is 6 "Evaluating Biological Plausibility in Supporting 7 Evidence For Action Through Systematic Reviews in 8 Public Health." 9 When is the first time you reviewed 10 this document, Doctor? 11 A In the last -- the last day or so. 12 Q Was the document provided to you by the 13 lawyers for plaintiffs? 14 A Yes. 15 Q The document is not referenced in your 16 report. True? 17 A It is not referenced. That's correct. 18 Q You did not review the document prior 19 to writing your report; correct? 20 A That's right. 21 Q The document was something the lawyers 22 for plaintiffs gave you after you had already 23 written and authored your report; correct? 24 A That's correct. I provided that as an</p>	<p style="text-align: right;">Page 144</p> <p>1 MS. O'DELL: 2 Object to the form. 3 A No, that's not true. 4 MS. BROWN: 5 Q The lawyers for plaintiffs found 6 Exhibit 14 in the scientific literature; correct? 7 A That's correct. 8 Q In reviewing the scientific literature, 9 did you pay attention to the articles that 10 classify different types of talcum powder 11 products? 12 MS. O'DELL: 13 Object to the form. 14 A Could you give a specific example, and 15 then I -- 16 I wouldn't be able to answer without 17 knowing. 18 MS. O'DELL: 19 Q Sure. 20 Do you understand that some of the talc 21 epidemiology separates use by type of talcum 22 powder product? 23 MS. O'DELL: 24 Objection to form.</p>
<p style="text-align: right;">Page 143</p> <p>1 example of the -- of a published example of the 2 methodology that I employed. 3 Q You didn't endeavor to research the 4 scientific literature to find a published -- 5 published example of your methodology, did you? 6 MS. O'DELL: 7 Objection to form. 8 A I -- it wasn't -- that wasn't what I 9 was -- I wasn't asked to reference the 10 methodology in my report. I was, again, asked to 11 provide an opinion on a biologically plausible 12 mechanism and then, since our discussion has 13 transferred to methodology, to be complete, I 14 wanted to provide an example of a published 15 version of the methodology that -- that is 16 similar to or at least describes in a summary or 17 really in that particular paper an exemplary 18 fashion of the criteria for biological 19 plausibility and the methods used therein. 20 MS. BROWN: 21 Q Exhibit 14 is the product of research 22 the lawyers for plaintiffs conducted on a 23 published article regarding your methodology. 24 True?</p>	<p style="text-align: right;">Page 145</p> <p>1 A Again, do you have a specific example 2 of one of the studies so I could -- so I'd be 3 able to accurately answer your question? 4 MS. BROWN: 5 Q Here's what I want to know. Did you 6 look at the studies that separated deodorizing 7 sprays from powder products from cornstarch, for 8 example? 9 A Certainly in my review I made as 10 comprehensive a review of available literature 11 as -- as possible. And, again, if you can name a 12 specific study or one of the references, I can 13 confirm if that was -- if that was part of 14 the -- my review of the epidemiology. 15 Q Do you hold the opinion that talcum 16 powder-containing deodorant sprays causes 17 inflammation? 18 MS. O'DELL: 19 Objection to form. Vague. 20 A So if the -- 21 Again, I was asked to provide an 22 opinion on the biologically plausible mechanism 23 regarding talc and talcum powder. So, 24 presumably, any product that contains talcum</p>

37 (Pages 142 to 145)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 146</p> <p>1 powder could possibly follow that same</p> <p>2 biologically plausible mechanism.</p> <p>3 MS. BROWN:</p> <p>4 Q Is there a certain amount of talcum</p> <p>5 powder that a product must contain to cause</p> <p>6 inflammation?</p> <p>7 MS. O'DELL:</p> <p>8 Objection to form.</p> <p>9 A That wasn't something I was asked</p> <p>10 to -- to quantify, similar to the discussions we</p> <p>11 had about metals, fragrances, and asbestos.</p> <p>12 MS. BROWN:</p> <p>13 Q In forming your opinion that talcum</p> <p>14 powder products cause inflammation, you have not</p> <p>15 attempted to quantify how much talcum powder is</p> <p>16 in those products; is that right?</p> <p>17 MS. O'DELL:</p> <p>18 Objection to form. Asked and answered.</p> <p>19 A So my -- my review included a number of</p> <p>20 studies that looked at exposure rates, and my</p> <p>21 review also included the review of some studies</p> <p>22 that did not include use frequency as well as use</p> <p>23 duration. And, so, both of those considerations</p> <p>24 in terms of my review of the epidemiology were</p>	<p style="text-align: right;">Page 148</p> <p>1 Objection to form. Vague.</p> <p>2 A My -- my opinions are based on the</p> <p>3 available scientific literature regarding the</p> <p>4 testing performed on talcum powder and talcum</p> <p>5 powder products.</p> <p>6 I -- in my review of those results, I</p> <p>7 did not see a specific enumeration of any one</p> <p>8 particular chemical composition that was -- had a</p> <p>9 greater or lesser cause or effect relationship.</p> <p>10 MS. BROWN:</p> <p>11 Q Do you know how much talcum powder is</p> <p>12 in the Shower to Shower product?</p> <p>13 A No. I wasn't -- I wasn't asked to</p> <p>14 quantify that, and I would defer to some of the</p> <p>15 other expert reports regarding the composition of</p> <p>16 those products.</p> <p>17 Q Do you include cornstarch as a talcum</p> <p>18 powder product?</p> <p>19 MS. O'DELL:</p> <p>20 Object to the form.</p> <p>21 A Cornstarch was included in some of the</p> <p>22 epidemiology studies, as you -- as you mentioned</p> <p>23 a moment ago.</p> <p>24 MS. BROWN:</p>
<p style="text-align: right;">Page 147</p> <p>1 undertaken, but I did not attempt to quantify</p> <p>2 those relationships specifically.</p> <p>3 MS. BROWN:</p> <p>4 Q Okay. So there's two different issues</p> <p>5 there that I want to ask you about. One, I want</p> <p>6 to talk to you about whether the talcum powder</p> <p>7 products you've described on page 17 of your</p> <p>8 report have a specific composition, in your mind.</p> <p>9 Okay?</p> <p>10 Two, I want to talk to you about what</p> <p>11 you were just answering, which is is there a</p> <p>12 specific amount of the product that you believe</p> <p>13 causes inflammation.</p> <p>14 Do you understand the difference?</p> <p>15 A I do.</p> <p>16 MS. O'DELL:</p> <p>17 Objection to form.</p> <p>18 MS. BROWN:</p> <p>19 Q Okay. So let's start, one, with the</p> <p>20 product. In forming the opinion that talcum</p> <p>21 powder products cause inflammation, is there a</p> <p>22 particular chemical composition that you are</p> <p>23 relying on?</p> <p>24 MS. O'DELL:</p>	<p style="text-align: right;">Page 149</p> <p>1 Q Do you consider cornstarch to be a</p> <p>2 talcum powder product that also causes</p> <p>3 inflammation?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form.</p> <p>6 A My -- my review of the literature</p> <p>7 doesn't -- I'm thinking through the available</p> <p>8 studies, and I don't recall which studies that</p> <p>9 may -- may have been a dependent variable in</p> <p>10 terms of the determination. So I -- I can't</p> <p>11 answer that. I -- I don't have the information</p> <p>12 to answer that accurately.</p> <p>13 MS. BROWN:</p> <p>14 Q So, sitting here today, you're not sure</p> <p>15 if cornstarch would be a talcum powder product</p> <p>16 that causes inflammation as you described on page</p> <p>17 17?</p> <p>18 MS. O'DELL:</p> <p>19 Objection.</p> <p>20 A No. So --</p> <p>21 MS. O'DELL:</p> <p>22 Misstates the testimony.</p> <p>23 But you may answer if you understand</p> <p>24 the question.</p>

38 (Pages 146 to 149)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 150</p> <p>1 A So corn -- cornstarch and -- and talcum 2 powder are -- are -- when I'm referring to talcum 3 powder and talcum powder products, cornstarch, as 4 a singular component -- or singular product, is 5 not included in that definition. 6 Now, whether products that contain talc 7 also contain cornstarch, I -- I'm not able to 8 say. 9 MS. BROWN: 10 Q Right. And so that's my question. 11 What about a product like Shower to Shower that 12 contains talc and cornstarch? How have 13 you -- what methodology have you employed to 14 arrive at the conclusion that the Shower to 15 Shower product causes inflammation? 16 MS. O'DELL: 17 Object to the form. 18 A So my -- what I was requested was to 19 write an opinion as to the, again, the 20 biologically plausible mechanism that exposure to 21 talc and its constituents can lead to 22 inflammation. 23 I wasn't asked to provide as to what 24 the minimum or maximum thresholds are of any</p>	<p style="text-align: right;">Page 152</p> <p>1 on knowledge of how much talcum powder is 2 actually in the product; correct? 3 MS. O'DELL: 4 Objection. Misstates his testimony. 5 A Again, not a -- it wasn't part of -- it 6 wasn't an opinion I was asked to provide. 7 The -- the only -- or, I should say, 8 a -- a study that looked at the -- summarizing 9 the epidemiology literature that I reviewed, some 10 of those studies had a duration and component as 11 far as general talcum powder and talcum powder 12 product use. 13 MS. BROWN: 14 Q And I want to -- 15 A I don't -- 16 MS. O'DELL: 17 Excuse me. Let him finish. 18 A I was -- I was going to say I don't 19 recall those quantitating the percentage of 20 talcum powder in a -- in a given product in the 21 study. 22 MS. BROWN: 23 Q Right. And, so, you're getting a 24 little into the second question, which I do want</p>
<p style="text-align: right;">Page 151</p> <p>1 product or of any component of that product or 2 constituent. 3 The information I was provided was the 4 analysis of products like Shower to Shower and 5 Johnson & Johnson's product, to evaluate the 6 spectrum of talc and asbestos contamination in 7 some of the constituent components, and then -- 8 and, therefore, develop an opinion as to 9 the -- whether or not that those products are 10 supported by the same mechanism that I developed 11 the opinion on, meaning they have the constituent 12 components to cause inflammation. 13 MS. BROWN: 14 Q You have not made a determination of a 15 particular amount of talcum powder that is 16 required to be in a product for it to cause 17 chronic inflammation; correct? 18 MS. O'DELL: 19 Object to the form. 20 A I wasn't asked to provide such an 21 opinion. 22 MS. BROWN: 23 Q Your opinion that talcum powder 24 products cause chronic inflammation is not based</p>	<p style="text-align: right;">Page 153</p> <p>1 to talk about, which is how much people are 2 exposed to. 3 But sticking with just what's in the 4 product, have you made a determination that there 5 is a threshold amount of talcum powder that is 6 required to be in a product before you can 7 conclude that that product will cause chronic 8 inflammation? 9 MS. O'DELL: 10 Objection to form. Asked and answered. 11 A I -- again, I wasn't asked to provide 12 that -- that threshold opinion. 13 MS. BROWN: 14 Q And understanding whether or not there 15 is a threshold of how much talcum powder has to 16 be in a product to cause inflammation is not 17 necessary for you to opine that talcum powder 18 products cause chronic inflammation? 19 MS. O'DELL: 20 Objection. Misstates his testimony. 21 A So my -- my use of the terminology 22 "talcum powder products" includes the product and 23 all of its constituent components, which would 24 be, as we earlier discussed, talcum powder,</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 154</p> <p>1 fragrances, and any contaminating substances, 2 such as asbestos or -- or heavy metals. 3 And, so, therefore, to -- to more -- to 4 answer -- to be able to answer your question 5 accurately, we would -- I think we would have to 6 have some discussions as to the type of talcum 7 powder and the level of exposure to be able to 8 answer that regarding my opinion in terms of 9 level. 10 You know, the -- to clarify, the -- 11 during this research and the -- and having the 12 opportunity to review much of the literature in 13 talcum powder, it's a -- it's a fascinating field 14 because it is similar to asbestos. It appears 15 that the diversity of products and the diversity 16 of talc sources are like having a thorn bush with 17 different size thorns, and, depending on the 18 constituent components, you know, those thorns 19 are bigger or smaller or otherwise. And -- but 20 my opinion is based on the fact that the presence 21 of any of those thorns is sufficient to cause 22 some inflammatory response. 23 MS. BROWN: 24 Q Does a talcum powder product with 10</p>	<p style="text-align: right;">Page 156</p> <p>1 exposure to inflammation to the initiation of 2 core progression of cancer. And that's -- that's 3 been the focus of my opinion. 4 MS. BROWN: 5 Q Have you attempted to quantify talc 6 exposure as it relates to individuals? 7 A No, I have not. 8 Again, my -- my opinions are primarily 9 limited to the -- to the biological mechanism. 10 Q Well, isn't that dependent, though, on 11 how much talc a person is exposed to? 12 MS. O'DELL: 13 Objection. 14 A No. Again, separating the -- so the 15 question of the mechanism is -- 16 Can an exposure result in a mechanism 17 is separate from how much of an exposure is 18 required to cause that mechanism. 19 MS. BROWN: 20 Q So you've identified two questions for 21 us. One, can exposure result in a mechanism. 22 Correct? 23 A (Nods affirmatively.) 24 Q And, two, how much of an exposure do</p>
<p style="text-align: right;">Page 155</p> <p>1 percent talc cause chronic inflammation, in your 2 view? 3 MS. O'DELL: 4 Object to the form. Incomplete 5 hypothetical. 6 A I -- I don't have the information to 7 answer that. 8 MS. BROWN: 9 Q Does a talcum powder product with 10 50 percent talc cause chronic inflammation, in 11 your view? 12 A Again, I don't have the information to 13 answer that. 14 MS. O'DELL: 15 Object to the form. 16 MS. BROWN: 17 Q Is it necessary for you to determine 18 the level of talc in a product before determining 19 that it can cause chronic inflammation? 20 MS. O'DELL: 21 Objection. Asked and answered. 22 A No. My -- my -- so my opinion was 23 asked to answer the question of can -- is there a 24 biologically plausible mechanism from talc</p>	<p style="text-align: right;">Page 157</p> <p>1 you need to produce a mechanism. Correct? 2 MS. O'DELL: 3 Objection to form. 4 A Correct. 5 MS. BROWN: 6 Q And, in this case, you have answered 7 question number one, can exposure to talc cause 8 chronic inflammation. Correct? 9 A So my -- yeah. My -- my report details 10 the -- that opinion regarding a biologically 11 plausible mechanism. 12 Q You have not, in this case, answered 13 question number two, which is how much exposure 14 to talc is needed to cause chronic inflammation. 15 Is that right? 16 MS. O'DELL: 17 Objection to form. 18 A I wasn't asked to provide such a 19 mechanism or such a -- such an opinion. 20 Part of my review included some of the 21 epidemiology studies that examine that question, 22 but I certainly would defer to the -- the number 23 of -- of epidemiologists who are -- who are 24 providing testimony in this case, rather than try</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 158</p> <p>1 and paraphrase or opine on their work. 2 MS. BROWN: 3 Q Do you believe -- 4 MS. O'DELL: 5 Excuse me. We've been going about an 6 hour and 15 minutes. I'd love to take a break in 7 the next two or three minutes and -- 8 MS. BROWN: 9 It will probably take me a little 10 longer than that, but I'm mindful of the time, 11 and I'll just finish this subject and take a 12 break -- 13 MS. O'DELL: 14 Well, Dr. Levy, would you like a break 15 now? 16 THE WITNESS: 17 I think we can finish this subject. 18 MS. BROWN: 19 Thank you. 20 THE WITNESS: 21 I -- I'd rather conclude it than break 22 it up. 23 MS. BROWN: 24 Q So, Doctor, as it relates to how much</p>	<p style="text-align: right;">Page 160</p> <p>1 epidemiology studies found that conclusion and, 2 as -- as reviewed in the report, you know, found 3 an increased risk with increasing -- increasing 4 exposure appears, with the current knowledge in 5 the literature, to increase risk. But my opinion 6 was not to further quantify or further describe 7 that. 8 MS. BROWN: 9 Q Many of the studies you looked at did 10 not show a dose response; correct? 11 MS. O'DELL: 12 Objection to form. 13 A The limitation of several of the 14 studies I reviewed was that they did not examine 15 a dose response, so that, therefore, the study 16 was unable -- unable to make that conclusion 17 because they didn't look. 18 MS. BROWN: 19 Q And some of the studies that did 20 attempt to look at duration and/or frequency did 21 not show a linear dose response. Correct? 22 A I would have to look at the specific 23 studies. But in -- in summary, studies that did 24 look at dose response, particularly more recent</p>
<p style="text-align: right;">Page 159</p> <p>1 talc is needed to cause inflammation that can 2 cause cancer, that wasn't what you were asked to 3 figure out in this case. Is that right? 4 MS. O'DELL: 5 Objection to form. 6 A No. Well, I -- I was -- I was asked to 7 provide a review of the literature in terms of 8 talc exposure and inflammation and, in that 9 review, identified a number of studies that 10 examined some relationships to dose. 11 But I -- as you -- as you see in my 12 conclusions, none of them speak to dose or 13 duration in terms of that -- of that mechanism. 14 MS. BROWN: 15 Q You are not offering an opinion in this 16 case, Doctor, that perineal use of talcum powder 17 exposes an individual to enough talc to cause 18 chronic inflammation than can cause cancer; 19 correct? 20 MS. O'DELL: 21 Objection to form. 22 A My review of studies that attempted to 23 answer that specific question found a -- or a 24 number of studies, both -- or a number of</p>	<p style="text-align: right;">Page 161</p> <p>1 studies with larger numbers of participants, the 2 meta-analysis studies, found a significant 3 relationship between duration of use as well as 4 frequency of use in terms of their -- their risk 5 ratios. 6 Q And you are not going to offer the 7 opinion in this case that a woman using Johnson's 8 Baby Powder products perineally is exposed to 9 enough talcum powder to cause chronic 10 inflammation that can cause cancer. True? 11 MS. O'DELL: 12 Object to the form. 13 A I -- I wasn't asked to -- to provide 14 that opinion. 15 MS. BROWN: 16 Q And so, as such, you haven't attempted 17 to quantify how much talcum powder, as used 18 perineally, might get to the ovary. Is that 19 fair? 20 A Again, wasn't -- wasn't asked. I was 21 able to review some of the literature that 22 is -- appears to be long -- longstanding, well 23 established over the last greater than 40 years 24 that show a clear -- and I believe the FDA</p>

41 (Pages 158 to 161)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 162</p> <p>1 statement is -- is describing it as inarguable -- 2 that talc can migrate either from perineal 3 exposure or even from inhalation exposure and be 4 found in the ovary. 5 A quantitation of how much exposure is 6 required for that migration to occur and -- or 7 how many times of exposure that migration needs 8 to occur, I think it's been a fairly wide 9 diversity of -- of studies on that subject. 10 And, so, based on that, I'm not able to 11 offer an opinion as to a minimal or maximum dose 12 required to get there, other than -- but, 13 instead, state that there is enough evidence to 14 say factually that migration through the -- or 15 through at least two mechanisms of exposure, talc 16 can be found in the ovary. And I would suggest 17 that -- or I'm not aware of any study that 18 quantitates that further. 19 Q Is it essential to your opinion that 20 talc causes chronic inflammation that can lead to 21 ovarian cancer that some amount of talc be 22 present in the actual ovary? 23 MS. O'DELL: 24 Object to the form.</p>	<p style="text-align: right;">Page 164</p> <p>1 talc has to reach the ovary for the chronic 2 inflammation to occur. Is that right? 3 MS. O'DELL: 4 Objection. 5 A Not -- specific to your question, 6 chronic inflammation, no, not necessarily. 7 MS. BROWN: 8 Q Is it your opinion in this case, 9 Doctor, that a woman can develop ovarian cancer 10 from chronic inflammation from talc without any 11 particle of talc ever reaching the ovary? 12 MS. O'DELL: 13 Objection to form. 14 A No, I didn't -- I -- I certainly did 15 not make that statement. And the -- 16 Again, restating the -- this summary of 17 my -- my opinion, that the biologically plausible 18 mechanism for talc exposure to inflammation to 19 cellular damage and then potentially creating the 20 correct environment is based on evidence showing 21 talc exposure in the ovary. 22 MS. BROWN: 23 Q Okay. So critical to your opinion, 24 then, some talc has to get to the ovary at some</p>
<p style="text-align: right;">Page 163</p> <p>1 A So my -- my -- my opinion regarding the 2 biologically plausible mechanism, again, does not 3 rely on duration of exposure or amount of 4 exposure. 5 So, therefore, I would -- I would 6 answer your question directly that it would be 7 no, it does not -- it would not necessarily 8 require talc to be present at the ovary at any 9 given time point for there to be the potential 10 that she had some inflammatory injury due to talc 11 exposure at a previous time. 12 That would, of course, be two different 13 questions, one being effect of exposure and 14 second question being is there clearance of that 15 exposure over time if use is discontinued. 16 So that's, again, two different -- two 17 very different scientific studies would be -- 18 would be necessary. 19 MS. BROWN: 20 Q And you have not undertaken either of 21 those studies. Is that fair? 22 A That's fair. 23 Q And -- but essential to your theory, 24 though, Doctor, at some point, some amount of</p>	<p style="text-align: right;">Page 165</p> <p>1 time; right? 2 A Well, the -- again, the -- my opinion 3 is not based on how talc migrates or -- or when 4 it can migrate. It's simply based on the, again, 5 that biological premise, that exposure to talc. 6 So I wasn't asked to opine whether or 7 not talc exposure in a neighboring tissue could 8 cause enough of an inflammatory response to 9 affect the ovary. 10 So there is the, certainly, the 11 uninvestigated secondary effects that perhaps 12 talc did not -- is not necessary or -- and 13 required to get to the ovary to cause that 14 effect. I'm -- I'm just not aware of any studies 15 that have made that delineation of talc exposure 16 to neighboring or surrounding organs. 17 There is limited or some suggestion 18 regarding the inflammatory response related to 19 talc exposure in the lung that suggests that any 20 talc exposure causes an inflammatory response. 21 Again, but I can't point you to evidence that 22 would take that inflammatory response and tie it 23 specifically to ovarian cancer. 24 So, again, my answer is there is not</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 166</p> <p>1 enough evidence to -- to support nor refute that</p> <p>2 any talc exposure can lead to an increased risk</p> <p>3 of ovarian cancer. What I do know from my review</p> <p>4 of the literature is the studies that looked at</p> <p>5 that specific exposure --</p> <p>6 And, to be clear, none of the</p> <p>7 epidemiology studies in humans quantitated the</p> <p>8 amount of talc reaching the ovary. It was simply</p> <p>9 the exposure and the -- and the perineal use of</p> <p>10 talc. So I think any discussion about how much</p> <p>11 did it reach the ovary and how long was it in the</p> <p>12 ovary is all hypothetical.</p> <p>13 Q Why don't we go off the record and take</p> <p>14 a break.</p> <p>15 Thank you, Doctor.</p> <p>16 VIDEOGRAPHER:</p> <p>17 Going off the record. The time is</p> <p>18 11:51 a m.</p> <p>19 (LUNCH RECESS.)</p> <p>20 VIDEOGRAPHER:</p> <p>21 We're back on the record. The time is</p> <p>22 12:52 p m.</p> <p>23 MS. BROWN:</p> <p>24 Q Welcome back, Doctor.</p>	<p style="text-align: right;">Page 168</p> <p>1 by well-established biological facts?</p> <p>2 A I would say the -- that chronic</p> <p>3 inflammation as a component of causing ovarian</p> <p>4 cancer is well established by biologically</p> <p>5 plausible facts.</p> <p>6 Q And what are those facts?</p> <p>7 A I think a number of studies that</p> <p>8 include the, first, the -- that talc or talcum</p> <p>9 powder causes inflammation. These exist in a</p> <p>10 number of forms, including very recent -- recent</p> <p>11 research by Dr. Saed, as we were -- touched on a</p> <p>12 little bit earlier in the -- in his paper, as</p> <p>13 well as classical studies with talc pleurodesis</p> <p>14 where there's -- you know, the fundamentals of</p> <p>15 that treatment is the inflammatory response</p> <p>16 caused by talc.</p> <p>17 Q Uh-huh.</p> <p>18 A And, so, that would be the -- some of</p> <p>19 the -- two examples of where factual information</p> <p>20 or at least observations that are supportive</p> <p>21 of -- of that information, you know, being</p> <p>22 considered as a bio- -- part of a biologically</p> <p>23 plausible mechanism.</p> <p>24 Q You would agree, Doctor, that not all</p>
<p style="text-align: right;">Page 167</p> <p>1 You were asked in this case to assess</p> <p>2 whether perineal use of talcum powder products</p> <p>3 induces a biologically plausible mechanism or</p> <p>4 mechanisms that result in ovarian cancer.</p> <p>5 Correct?</p> <p>6 A Correct.</p> <p>7 Q And define for us, if you will,</p> <p>8 "biologically plausible mechanism" as you used it</p> <p>9 in that sentence.</p> <p>10 A Excuse me. A mechanism that is</p> <p>11 biologically plausible, I mean that it is</p> <p>12 supported by either well-established biological</p> <p>13 facts or supported by at least a single line of</p> <p>14 evidence in published literature -- you know,</p> <p>15 generally speaking, peer-reviewed literature but</p> <p>16 certainly not limited to that -- where when you</p> <p>17 take -- when you consider the totality of the</p> <p>18 mechanism, that, essentially, each of the steps</p> <p>19 makes sense and is -- is supported by -- through</p> <p>20 either direct or indirect observations.</p> <p>21 Q Okay. And, in this case, as it relates</p> <p>22 to talcum powder, do you believe that the</p> <p>23 biologically plausible mechanism of chronic</p> <p>24 inflammation causing ovarian cancer is supported</p>	<p style="text-align: right;">Page 169</p> <p>1 inflammation causes cancer; correct?</p> <p>2 A I would say inflammation is not</p> <p>3 singularly responsible for cancer. However, I</p> <p>4 would clarify that the progression from cellular</p> <p>5 transformation to malignant cancer, at least with</p> <p>6 our current understanding of cancer biology,</p> <p>7 appears to have an inflammatory requirement,</p> <p>8 meaning that all cases of chronic inflammation</p> <p>9 don't necessarily cause cancer. However, our</p> <p>10 understanding of malignant transformation appears</p> <p>11 to have, universally, an inflammatory component.</p> <p>12 Q Okay. You would agree, though, that</p> <p>13 not all types of inflammation that the body</p> <p>14 experiences is inflammation that will lead to</p> <p>15 cancer. Correct?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A So I would -- taking a step back</p> <p>19 and -- and -- or to orient us to some of the</p> <p>20 basis of my opinions and some statements on</p> <p>21 general cancer biology --</p> <p>22 MS. BROWN:</p> <p>23 Q Well, let's start with just the</p> <p>24 question, though, Doctor.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 170</p> <p>1 A Okay.</p> <p>2 Q Okay. Let's just keep it to an answer</p> <p>3 to the question. And then if you need an</p> <p>4 opportunity to make another statement on the</p> <p>5 record, that's fine.</p> <p>6 MS. O'DELL:</p> <p>7 Excuse me. Just object to the</p> <p>8 direction of the witness.</p> <p>9 Dr. Levy, you can answer a question</p> <p>10 however you'd like.</p> <p>11 MS. BROWN:</p> <p>12 Q And, just to orient you, Doctor, what</p> <p>13 I'm after, the question was: Not all</p> <p>14 inflammation that takes place in the body is</p> <p>15 inflammation that leads to cancer; correct?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A So that, yeah, it's really too general</p> <p>19 a question. So you're -- you're -- what you're</p> <p>20 asking is does all inflammation have the</p> <p>21 potential to have -- have a relationship to</p> <p>22 cancer, and the answer to that is -- is yes, it</p> <p>23 does.</p> <p>24 Now, does every inflammatory response</p>	<p style="text-align: right;">Page 172</p> <p>1 cause cancer. The -- you need a contribution of</p> <p>2 other factors. And what those factors are is --</p> <p>3 some are understood. Some are areas of active</p> <p>4 research.</p> <p>5 In the -- in the specific case of</p> <p>6 ovarian cancer, it does appear, given the</p> <p>7 late- -- given the observations about latency</p> <p>8 period, that some level of chronic inflammation</p> <p>9 appears to be critical, but there is no</p> <p>10 definition of it being required to then having</p> <p>11 acute inflammation, again, in summary, causing</p> <p>12 cellular damage and then chronic inflammation</p> <p>13 providing a -- a supportive environment for that</p> <p>14 transformation.</p> <p>15 And, again, I'm -- I'm generalizing,</p> <p>16 which, as we discussed earlier in the day, cancer</p> <p>17 is very complex, and so we have to be cautious</p> <p>18 with generalizations.</p> <p>19 Q Talc pleurodesis is a medical procedure</p> <p>20 by which talc is injected into the pleura;</p> <p>21 correct?</p> <p>22 A Correct.</p> <p>23 Q And it is done that purposefully to</p> <p>24 elicit an inflammatory response. Correct?</p>
<p style="text-align: right;">Page 171</p> <p>1 directly cause cancer? And that's a question</p> <p>2 that I would say would be reasonable to -- in</p> <p>3 layperson's terms, in terms of general</p> <p>4 inflammation, is unlikely.</p> <p>5 But there -- their distinction</p> <p>6 between -- is -- you know, stated simply, is</p> <p>7 inflammation is a -- by our current knowledge of</p> <p>8 cancer, is a necessary component of cancer</p> <p>9 progression. That does not equate to all</p> <p>10 inflammation causing cancer.</p> <p>11 MS. BROWN:</p> <p>12 Q Does acute inflammation cause cancer,</p> <p>13 in your mind, Doctor?</p> <p>14 A It is a component of the cancer</p> <p>15 progression process. And, so, in my -- to</p> <p>16 provide a simplistic distinction between them is</p> <p>17 a --</p> <p>18 Acute inflammation which results in</p> <p>19 either an inflammatory response or direct</p> <p>20 cellular insult or injury can be viewed as having</p> <p>21 a -- causing cellular damage that results</p> <p>22 in -- in cellular transformation.</p> <p>23 Now, that is not sufficient for that --</p> <p>24 for those transformed cells to then go on to</p>	<p style="text-align: right;">Page 173</p> <p>1 A That's correct.</p> <p>2 Q And have you looked in consid- --</p> <p>3 forming your opinions in this case at the body of</p> <p>4 epidemiology that has followed folks who received</p> <p>5 talc pleurodesis to see if they developed cancer?</p> <p>6 MS. O'DELL:</p> <p>7 Object.</p> <p>8 A Somewhat, yes.</p> <p>9 MS. BROWN:</p> <p>10 Q And are you familiar with the findings</p> <p>11 of those studies that talc, when injected</p> <p>12 directly into the pleura for the purpose of</p> <p>13 causing inflammation, had not caused cancer?</p> <p>14 MS. O'DELL:</p> <p>15 Object to the form.</p> <p>16 A I would disagree with your conclusions.</p> <p>17 And, in fact, the literature I reviewed has, I</p> <p>18 think, two fundamental concerns. One is the time</p> <p>19 period that these patients were followed post</p> <p>20 pleurodesis, and the other that there -- there</p> <p>21 have been at least one report, perhaps two -- I</p> <p>22 would have to review to make sure I'm speaking</p> <p>23 accurately -- where there was indeed a</p> <p>24 asbestos-like response in the formation of a</p>

44 (Pages 170 to 173)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 174</p> <p>1 mesothelioma-like event in the -- in the -- in 2 the pleural space following talc pleurodesis. 3 However, you know, taking a step back, 4 given the relative rarity of that as a procedure, 5 particularly today, I think drawing conclusions 6 from that as its -- as its relationship to cancer 7 would be difficult, but I -- I do think 8 fundamentally the -- my use of that as an example 9 was not necessarily to tie talc specifically to 10 cancer. It was more to state that it's well 11 established that platy talc individually as it -- 12 used in those procedures causes an inflammatory 13 response. And so, you know -- and that is the 14 primary reason I used or reviewed that literature 15 for that purpose. 16 MS. BROWN: 17 Q Is it your opinion, Doctor, that talc 18 pleurodesis leads to cancer? 19 MS. O'DELL: 20 Object to the form. 21 A It is my opinion that talc pleurodesis 22 creates an environment supportive of cancer. And 23 whether or not some number of individuals may 24 progress, could progress or have progressed to</p>	<p style="text-align: right;">Page 176</p> <p>1 mid-'80s to early '90s. I'd have to, again, have 2 to review that -- 3 I gave that specific example of a 4 patient or cohort of patients that were found to 5 have, again, asbestos-like effects in the lung 6 leading to, at least in a case or more than 7 perhaps more than one case, a mesothelioma-like 8 effect like we -- like I just mentioned. 9 But, again, to point you to the exact 10 reference, I'd have to review. 11 MS. BROWN: 12 Q Are you relying on that reference in 13 forming your opinions in this case? 14 A No. Specifically -- again, to restate 15 the -- my description of the pleurodesis process 16 was to support the early part of the biological 17 mechanism that talc causes inflammation. So 18 that -- and, so, in the lung as a tissue, that 19 progression to cancer is -- is -- I think is a -- 20 is a -- is a supportive observation to the -- to 21 my overall principle. But, again, it's a 22 separate -- separate exposure type, certainly a 23 very different dosing, potentially, and, again, a 24 very different patient, or the patient is a very</p>
<p style="text-align: right;">Page 175</p> <p>1 cancer is -- you know, is -- is of limited 2 knowledge right now. 3 MS. BROWN: 4 Q What scientific support do you have for 5 your opinion that talc pleurodesis creates an 6 environment supportive of cancer? 7 A Oh, just that it causes an inflammatory 8 response. And, as we've been discussing, there 9 is ample evidence surrounding the role of 10 inflammation in cancer. There's a -- you know, 11 in a number of both reference studies and I think 12 generally, I would -- I would state that it's a 13 generally accepted fact in cancer biology. 14 Q What scientific support do you have for 15 your opinion that talc pleurodesis patients later 16 can and do develop cancer? 17 MS. O'DELL: 18 Object to the form. Misstate his 19 testimony. 20 A I'd have to review my -- review some of 21 the literature. And I can take a look if we want 22 to pause for a moment. 23 But there was -- I recall one study 24 involving talc pleurodesis that was maybe</p>	<p style="text-align: right;">Page 177</p> <p>1 different individual in the sense that they 2 obviously have reasons for going through the talc 3 pleurodesis which are -- which are -- which are 4 potentially compounding to the overall phenotype. 5 Q Have you endeavored to quantify the 6 difference between exposure to talc from 7 pleurodesis versus perineal use of cosmetic 8 talcum powder products? 9 MS. O'DELL: 10 Object to the form. 11 A I have -- I have not attempted to 12 delineate those two simply from the perspective 13 that, again, to the biological mechanism, the 14 initial premise is talc causes inflammation. And 15 when I examined literature to look for evidence 16 of that historically, talc pleurodesis is one 17 example of inflammation. There's now others, and 18 there's, subsequent to that, there's been 19 a -- now a number of -- or, you know, probably 20 a -- 21 Dr. Saed is one example of a reasonably 22 comprehensive molecular study examining specific 23 inflammatory markers tied specifically to 24 cellular exposure to, in the case of that paper,</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 178</p> <p>1 specific products, you know, such as the Shower</p> <p>2 to Shower and the -- and baby powder.</p> <p>3 MS. BROWN:</p> <p>4 Q Do you believe the inflammation caused</p> <p>5 by talc pleurodesis is chronic inflammation that</p> <p>6 leads to cancer?</p> <p>7 MS. O'DELL:</p> <p>8 Objection to form. Asked and answered.</p> <p>9 A Again, I believe the inflammatory</p> <p>10 response to talc exposure, which would include</p> <p>11 talc pleurodesis, induces an inflammatory</p> <p>12 response that would be supportive of cancer</p> <p>13 development and/or progression.</p> <p>14 MS. BROWN:</p> <p>15 Q And what scientific literature other</p> <p>16 than the one study you just referenced for us do</p> <p>17 you rely on for your opinion that talc</p> <p>18 pleurodesis induces an inflammatory response that</p> <p>19 would be supportive of cancer development and/or</p> <p>20 progression?</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A All my -- my opinion is based on</p> <p>24 connecting two basic concepts. Talc exposure</p>	<p style="text-align: right;">Page 180</p> <p>1 powder products cause chronic inflammation in</p> <p>2 your November 2018 report before having seen the</p> <p>3 Saed paper from 2018; correct?</p> <p>4 MS. O'DELL:</p> <p>5 Object -- object to the form.</p> <p>6 Misstates his testimony.</p> <p>7 A The -- so, as we discussed -- we</p> <p>8 discussed earlier, I had seen abstract</p> <p>9 information as well as earlier publication from</p> <p>10 Dr. Saed's group and that the current 2018 paper,</p> <p>11 while not necessary for the opinions described in</p> <p>12 the report, certainly support those opinions,</p> <p>13 given that it was a direct assessment of specific</p> <p>14 products, specific -- in specific doses applied</p> <p>15 to cellular material and then measurements for</p> <p>16 inflammation made directly on that material.</p> <p>17 So while that particular study was</p> <p>18 not --</p> <p>19 And, again, the -- the earlier studies</p> <p>20 that were used to inform the 2018 paper were</p> <p>21 certainly used in this report and referenced</p> <p>22 the --</p> <p>23 And I'm just recalling when. Or if</p> <p>24 we've refer- -- had the opportunity to reference</p>
<p style="text-align: right;">Page 179</p> <p>1 causes inflammation. Inflammation has a</p> <p>2 significant role in cancer development.</p> <p>3 And, so, as far as -- each of those is</p> <p>4 supported by individual -- individual studies,</p> <p>5 and -- and now -- as I mentioned, there are now</p> <p>6 studies that directly tie those together in</p> <p>7 observation.</p> <p>8 MS. BROWN:</p> <p>9 Q What is the scientific basis for your</p> <p>10 support that talc exposure causes the type of</p> <p>11 inflammation that has been linked to cancer?</p> <p>12 A The most recent is the Saed publication</p> <p>13 that we discussed and -- or at least has been</p> <p>14 mentioned. In that study, looking at -- there</p> <p>15 was a assessment and, in some cases, a</p> <p>16 quantitation of the specific molecular markers</p> <p>17 for inflammation that were induced, and many</p> <p>18 of -- some of those markers are shared with known</p> <p>19 markers for -- for cancer progression, such as</p> <p>20 CA 125, as well as others.</p> <p>21 Q Are you referring to Saed's 2018 paper,</p> <p>22 Dr. Levy?</p> <p>23 A Yes.</p> <p>24 Q And you formed the opinions that talcum</p>	<p style="text-align: right;">Page 181</p> <p>1 the --</p> <p>2 Yeah. So we reference primarily the</p> <p>3 abstracts and then, again, as well as some of the</p> <p>4 other Saed work, which is the foundation of the</p> <p>5 directed studies that are described in the</p> <p>6 Reproductive Sciences paper that is Exhibit 12.</p> <p>7 MS. BROWN:</p> <p>8 Q Do you know that Dr. Saed is a paid</p> <p>9 expert for the plaintiffs' lawyers in this</p> <p>10 litigation?</p> <p>11 A I am aware. Yes.</p> <p>12 Q Have you considered that fact in</p> <p>13 evaluating Dr. Saed's work?</p> <p>14 A I did.</p> <p>15 Q Other than Dr. Saed's work from 2017</p> <p>16 and 2018, what evidence are you relying on to</p> <p>17 support your opinion that talcum powder produces</p> <p>18 the type of inflammation that can lead to cancer?</p> <p>19 A There has been -- looking through</p> <p>20 the -- there's the Buz'Zard and Lau, 2007. We</p> <p>21 were discussing the Hamilton -- Hamilton paper in</p> <p>22 terms of immune response but then, more</p> <p>23 specifically, the NTP reference in 1993. And in</p> <p>24 those cases, that was either looking at increases</p>

46 (Pages 178 to 181)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 182</p> <p>1 in reactive oxygen species generation --</p> <p>2 THE COURT REPORTER:</p> <p>3 Wait a minute. You have to slow down</p> <p>4 when you read, please.</p> <p>5 MS. O'DELL:</p> <p>6 You may continue.</p> <p>7 A Just to -- before I left off, I think,</p> <p>8 in those mentioned references, the reactive</p> <p>9 oxygen species generation, increased cell</p> <p>10 proliferation, and the use of -- in the specific</p> <p>11 case of Buz'Zard and Lau, was looking at the</p> <p>12 transformation in human ovarian cancer cells that</p> <p>13 were treated with talcum powder -- sorry -- human</p> <p>14 ovarian cells treated with talcum powder.</p> <p>15 MS. BROWN:</p> <p>16 Q Other than Buz'Zard, Hamilton, and NTP,</p> <p>17 is there anything else that you are relying on to</p> <p>18 support your opinion that the inflammation caused</p> <p>19 by talcum powder is the type of inflammation that</p> <p>20 causes cancer?</p> <p>21 A So there's additional references</p> <p>22 mentioned in the report; Gates, Belot, Harper and</p> <p>23 Saed. And then, in addition to that, there was</p> <p>24 a --</p>	<p style="text-align: right;">Page 184</p> <p>1 the details, and I -- there -- I am aware</p> <p>2 of -- mentioned earlier the Woodruff or Woodford,</p> <p>3 the earlier 1971 paper where I couldn't remember</p> <p>4 the author, is one of the earliest studies that I</p> <p>5 came across that had -- it has an animal model</p> <p>6 study.</p> <p>7 MS. BROWN:</p> <p>8 Q Doctor, is it your testimony that --</p> <p>9 First of all, do you think it's -- that</p> <p>10 in opining that there is a biologically plausible</p> <p>11 mechanism by which talcum powder causes chronic</p> <p>12 inflammation that can cause ovarian cancer, is it</p> <p>13 necessary, in your mind, to be able to show in</p> <p>14 animals that talcum powder does just that?</p> <p>15 A That talcum powder causes inflammation?</p> <p>16 Q That causes ovarian cancer.</p> <p>17 A No, I don't -- I don't think that</p> <p>18 that's -- that's certainly not a requirement.</p> <p>19 And the reason I -- the reason I give that answer</p> <p>20 is -- is quite simple; that there is a wide</p> <p>21 diversity of animal model studies that have not</p> <p>22 been able to mimic specifically or correctly</p> <p>23 human cancer for both -- both from a detection</p> <p>24 and most often from a treatment perspective,</p>
<p style="text-align: right;">Page 183</p> <p>1 Make sure I'm referring to the right</p> <p>2 one.</p> <p>3 So those were the -- those were the</p> <p>4 primary references. And then, of course, there</p> <p>5 were supporting materials and other earlier-cited</p> <p>6 work.</p> <p>7 But for the opinion regarding the type</p> <p>8 of inflammation that is caused by exposure to</p> <p>9 talc and as far as its specific relationship to</p> <p>10 cancer, there's -- there's -- I would point to</p> <p>11 the, at least in the Saed work, the specific</p> <p>12 quantitation of a very well-known tumor marker,</p> <p>13 CA 125, also known as mucin-16 elevation in that</p> <p>14 work, and then, in the case of Gates, some of the</p> <p>15 fundamental glutathione S-transferase has been</p> <p>16 associated or has been observed as a higher risk.</p> <p>17 And, so, that would -- those would be</p> <p>18 some examples.</p> <p>19 Q Are you aware of any animal study,</p> <p>20 Dr. Levy, that shows the inflammation caused by</p> <p>21 talcum powder causing precancerous changes?</p> <p>22 MS. O'DELL:</p> <p>23 Object to the form.</p> <p>24 A I would have to review the -- a few of</p>	<p style="text-align: right;">Page 185</p> <p>1 meaning that, fundamentally, humans and most --</p> <p>2 or at least the animal systems used as -- in</p> <p>3 scientific modeling are different. Some of their</p> <p>4 differences are due to different pathways, and</p> <p>5 others of the differences are due to actually,</p> <p>6 you know, fundamental immune system differences.</p> <p>7 Q The Hamilton article that you</p> <p>8 identified for me, we marked earlier in the</p> <p>9 deposition as Exhibit 7. Do you recall that?</p> <p>10 MS. O'DELL:</p> <p>11 Counsel, would you mind just placing</p> <p>12 the exhibits by the witness so he can refer to</p> <p>13 them as he'd like, please.</p> <p>14 A Yes, I recall this.</p> <p>15 MS. BROWN:</p> <p>16 Q And you would agree with me, Doctor,</p> <p>17 that the Hamilton study that we discussed this</p> <p>18 morning concluded that there were no neoplastic</p> <p>19 changes in the animals that were injected with</p> <p>20 talcum powder; correct?</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form. Asked and</p> <p>23 answered.</p> <p>24 A No. No, I -- I wouldn't agree.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 186</p> <p>1 MS. BROWN: 2 Q What evidence in Hamilton, Doctor, are 3 you relying on to support your position that 4 Hamilton showed neoplastic changes in animals 5 injected with talc? 6 A Well, I'm not -- I'm not stating that 7 Hamilton specifically showed that. 8 What I'm stating is that -- that there 9 is a Hamilton study as an animal model system to 10 make the conclusion that, in this animal model 11 system, that talc or talcum powder does not -- or 12 that causes or does not cause ovarian cancer is 13 not -- it's -- it is -- it has limitations. 14 And, as we discussed a bit earlier, the 15 two limitations are the very limited time points 16 of the animals. And if we look at the relative 17 and observed time points that we know now, as far 18 as latency period, these are well short of 19 those -- of those periods, even by rat standards, 20 and then the number of treated animals is 21 relatively small at ten. So the... 22 Q Doctor, do you rely on the Hamilton 23 article to support your opinion that talcum 24 powder produces chronic inflammation that causes</p>	<p style="text-align: right;">Page 188</p> <p>1 Q So this article looked at talc that was 2 injected into animals and found no evidence of 3 changes that lead to cancer. Correct? 4 MS. O'DELL: 5 Objection to form. 6 A Over the time period that they -- that 7 the study was performed, they did -- they did 8 not -- they did not report, and, in fact, as you 9 said, their statements are "no evidence of 10 cellular atypia or mitotic activity." 11 MS. BROWN: 12 Q So in opining, as you do in this case, 13 that talcum powder can biologically induce 14 chronic inflammation that causes ovarian cancer, 15 what methodology did you employ to consider the 16 findings of the Hamilton article? 17 A Well, I considered the findings of the 18 Hamilton article, as -- as referenced in the 19 report, primarily showing that talc has an 20 inflammatory or an immune response. And that was 21 the primary inclusion of the -- of the Hamilton 22 paper. 23 Q Not all inflammatory or immune 24 responses lead to cancer; right?</p>
<p style="text-align: right;">Page 187</p> <p>1 ovarian cancer? 2 A No, I don't rely -- again, I don't rely 3 on any -- there's not a reliance on any singular 4 article. 5 Q Did not mean to suggest that, Doctor. 6 I asked you for the scientific support 7 that you have for the opinions you're giving in 8 this litigation, and one of the articles you 9 identified was the Hamilton article. Correct? 10 A Uh-huh. Yes. 11 Q And I -- and this Hamilton article, as 12 we discussed, at page 103, found no evidence of 13 neoplasm in the rats injected with talc. Right? 14 A They -- I -- I don't -- they did 15 not -- I don't recall seeing a description of 16 neoplasm in the Hamilton article. 17 Q Page 103, second column, begins with 18 "No evidence." 19 A "No evidence of cellular atypia." 20 Q Uh-huh. "And concludes that in no 21 ovary was there any evidence of frank neoplasia"; 22 right? 23 A Yes. That's what's written in the 24 paper.</p>	<p style="text-align: right;">Page 189</p> <p>1 MS. O'DELL: 2 Objection. Asked and answered. 3 A As -- as we discussed, not -- not all 4 inflammatory responses have been shown to 5 conclusively lead to cancer. And, so... 6 MS. BROWN: 7 Q And Hamilton does not support the 8 opinion that the type of inflammatory response 9 that talc causes is the type that causes cancer. 10 Fair enough? 11 MS. O'DELL: 12 Object to the form. 13 A No. I would say that's unfair. 14 Because, again, the limitation of the Hamilton 15 study at the time it was performed was -- is a 16 very short timeline. So there is -- it is an 17 incomplete study in the sense that there is 18 certainly the possibility that the first aspect 19 or the first event that we're -- that we've been 20 discussing in cancer biology, the cellular damage 21 to lead to transformation, could have occurred in 22 some of the rat tissues but had not progressed 23 enough or had -- or had taken hold enough to 24 cause or to have that be detected in this</p>

Shawn Levy, Ph.D.

Page 190	Page 192
<p>1 particular study performed in the early '80s.</p> <p>2 And, furthermore, rat -- the rat model</p> <p>3 for human cancer, since this study has been in</p> <p>4 other cases, has some limitations as it relates</p> <p>5 to how applicable it is to the human condition.</p> <p>6 MS. BROWN:</p> <p>7 Q The NTP study that you identified as</p> <p>8 supporting your opinion, Doctor, that also does</p> <p>9 not show evidence of neoplastic changes; is that</p> <p>10 right?</p> <p>11 MS. O'DELL:</p> <p>12 Object to the form.</p> <p>13 Doctor, please feel free to refer to</p> <p>14 the study if you need to.</p> <p>15 A Yeah. I'll do that now.</p> <p>16 (DEPOSITION EXHIBIT NUMBER 15</p> <p>17 WAS MARKED FOR IDENTIFICATION.)</p> <p>18 MS. BROWN:</p> <p>19 Q Doctor, we'll mark as Exhibit 15 to</p> <p>20 your deposition the NTP study to which you were</p> <p>21 referring.</p> <p>22 A Uh-huh.</p> <p>23 Q And this study, as well, does not show</p> <p>24 evidence of neoplastic changes.</p>	<p>1 Q Did you review, Doctor, the --</p> <p>2 And -- and what about the findings of</p> <p>3 NTP support your opinion?</p> <p>4 A Well, first, the inflammatory response,</p> <p>5 given the evidence by the accumulation of</p> <p>6 macrophages, and then, secondly, that in the</p> <p>7 female rats, the incidences of alveolar and</p> <p>8 bronchial or adenoma, carcinoma, and adenoma in</p> <p>9 the 18-milligram-per-meter group were</p> <p>10 significantly greater than those of controls.</p> <p>11 Q So did you consider the FDA's findings</p> <p>12 as it relates to the evaluation of the NTP study?</p> <p>13 MS. O'DELL:</p> <p>14 Object to the form. Vague.</p> <p>15 A Which -- which FDA?</p> <p>16 MS. BROWN:</p> <p>17 Q Have you considered, in connection with</p> <p>18 this case, the FDA's response to the 2014</p> <p>19 citizens petition?</p> <p>20 A Yes. That's familiar. And if I recall</p> <p>21 correctly --</p> <p>22 Or do you have -- is that handy?</p> <p>23 Q We'll mark that as Exhibit 16, Doctor.</p> <p>24 (DEPOSITION EXHIBIT NUMBER 16</p>
Page 191	Page 193
<p>1 MS. O'DELL:</p> <p>2 Object to the form.</p> <p>3 Do you have a copy for me?</p> <p>4 It's what number?</p> <p>5 MS. BROWN:</p> <p>6 Fifteen.</p> <p>7 A I think the -- the important</p> <p>8 distinction in this particular study is this was</p> <p>9 an aerosol-based -- based study. It certainly</p> <p>10 was longer than the Hamilton but was -- was not a</p> <p>11 study that mimics the perineal use of talc.</p> <p>12 MS. BROWN:</p> <p>13 Q And, so, as it relates to your opinion</p> <p>14 in this case, Doctor, that talc induces a chronic</p> <p>15 inflammation that can lead to ovarian cancer, the</p> <p>16 NTP study does not support that, does it?</p> <p>17 MS. O'DELL:</p> <p>18 Object to the form.</p> <p>19 A I would say the study does support my</p> <p>20 opinion regarding talc and its role in</p> <p>21 inflammation. And if we refer to page 6 within</p> <p>22 the first -- the first paragraph, beginning with</p> <p>23 "Accumulations of macrophages."</p> <p>24 MS. BROWN:</p>	<p>1 WAS MARKED FOR IDENTIFICATION.)</p> <p>2 MS. BROWN:</p> <p>3 Q The reason I want to talk to you about</p> <p>4 this is it contains a review of the NTP study we</p> <p>5 were just discussing.</p> <p>6 First of all, did you consider this</p> <p>7 document in connection with your opinions in this</p> <p>8 case?</p> <p>9 A Yes, this document's familiar.</p> <p>10 Q Okay. And do you recall that a cancer</p> <p>11 prevention coalition wrote the FDA requesting</p> <p>12 that a warning label be placed on talcum powder</p> <p>13 products?</p> <p>14 A Yes.</p> <p>15 Q And do you recall, as evidenced on</p> <p>16 page 1, the FDA reviewed the data as it related</p> <p>17 to that question?</p> <p>18 A I -- I recall that the FDA reviewed the</p> <p>19 data and determined that it was insufficient, and</p> <p>20 they did not identify any new compelling</p> <p>21 literature at the time. But this was in 2014.</p> <p>22 Q And the NTP --</p> <p>23 MS. O'DELL:</p> <p>24 Excuse me, counsel.</p>

49 (Pages 190 to 193)

Shawn Levy, Ph.D.

Page 194	Page 196
<p>1 Were you finished? If you're finished, 2 that's fine. I just didn't know if you completed 3 your -- 4 A I'm just reading. There was one 5 other -- I recall -- 6 MS. BROWN: 7 Q Doctor, the NTP study that you pointed 8 us to was from 1993. Is that right? 9 A I believe that's correct. 10 Q All right. And one of the things that 11 the FDA did in this letter of 2014 is reviewed 12 that study; correct? 13 A Yes. 14 Q And I'll direct you to page 3 of 7. 15 And what the FDA concluded was that the study 16 lacked convincing scientific support because of 17 serious flaws in its design and conduct. 18 Do you see that? 19 MS. O'DELL: 20 Where are you reading? Sorry. 21 MS. BROWN: 22 Page 3. Page 3. 23 MS. O'DELL: 24 Oh. Page 3. Sorry. I thought you</p>	<p>1 the FDA claimed serious flaws. 2 MS. BROWN: 3 Q At the bottom of page 3 -- 4 A I see. 5 Q -- the sentence that begins, "However, 6 this study lacks convincing scientific support 7 because of serious flaws in its design and 8 conduct -- and conduct." 9 Do you see that? 10 A I do. 11 Q And one of the things the FDA points to 12 is that the investigators used micronized talc 13 instead of consumer grade talc, resulting in the 14 experimental protocol not being reflective of 15 human exposure conditions in terms of particle 16 size. 17 Do you see that? 18 A I do. 19 Q Have you made a determination in this 20 case, sir, about the size of the particles in 21 talcum powder products? 22 A I -- I've not made that distinction. 23 And -- 24 Q There's --</p>
Page 195	Page 197
<p>1 said page 2. I'm sorry. 2 MS. BROWN: 3 Q Do you see that, Doctor? 4 A Starting with -- 5 Q Bottom of page 3 -- 6 A -- under toxicology findings? 7 Q So, to orient us here, Doctor, you 8 pointed, as evidence of support of your opinions 9 in this case, to the NTP study. Right? 10 A Correct. 11 Q And the folks who wrote to the FDA 12 requesting a warning on talc, they, too, pointed 13 to that study; right? 14 A Yes. 15 Q All right. And, so, the FDA reviewed 16 that study and, in the letter denying the 17 citizens petition, included its critique of that 18 study; correct? 19 A Correct. 20 Q And one of the things the FDA concluded 21 was that the study had serious flaws. True? 22 MS. O'DELL: 23 Objection to form. 24 A I don't -- do you -- I don't see where</p>	<p>1 A And, furthermore, I think the -- 2 importantly, the -- the flaws that the FDA points 3 out are, you know, not in disagreement with 4 our -- with our discussions surrounding both the 5 inflammatory response and then some of the 6 results there. I don't -- I don't see as a 7 concern -- 8 In fact, the -- it appears the FDA does 9 not disagree with the observation of the evidence 10 of carcinogenic activity in the non-asbestiform 11 talc. I think they -- 12 I share -- 13 Q Let's focus back on the question, 14 Doctor. 15 MS. O'DELL: 16 Excuse me. Let him finish his answer. 17 He's not finished. 18 A So, the, you know, the serious flaws 19 were the, I think, in this case, the specific 20 inclusion of nonasbestos talc and use of 21 micronized talc instead of consumer grade. So I 22 think in that -- in that sense, it's not 23 surprising that it had a different -- perhaps a 24 different response than may be observed with</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 198</p> <p>1 consumer products or talc that have -- may have 2 contaminants, whether it be asbestos or other. 3 MS. BROWN: 4 Q Do you remember the question I asked, 5 Doctor? 6 A Perhaps it would be helpful to restate. 7 Q I think, probably. 8 I asked if you had made a determination 9 in this case about the size of the particles in 10 talcum powder products. 11 A I -- so as far -- a determination, no. 12 I would -- I would say I have had an opportunity 13 to, you know, review or become more educated in 14 the diversity of talc products and the 15 interesting geographic relationship to different 16 size particles and -- in the presence or absence 17 of asbestiform particles in talc, which was a, 18 you know, fascinating area to become educated in. 19 As far as examining that in each of the 20 individual studies, I certainly was able to pay 21 attention to earlier or later studies as it 22 applied to when there was a specific description 23 of the talc, such as in the NTP study where 24 there -- that was one of the few that had a</p>	<p style="text-align: right;">Page 200</p> <p>1 when -- when used in the perineum compared to 2 inhalation. 3 But I have not seen a study that tried 4 to distinguish that in terms of having an exposed 5 group who inhaled talc only and then looked for 6 evidence of the presence in the ovary. 7 Q Back to the FDA document we were 8 discussing, Doctor, the FDA's critique of the NTP 9 study continues on page 4, where the FDA 10 identifies that the investigators conceded they 11 have problems with the aerosol generation system 12 and that the study did not include positive and 13 negative dust controls. 14 Did you consider those critiques in 15 evaluating the NTP study in this case? 16 MS. O'DELL: 17 Object to the form. 18 A Well, I -- I certainly considered -- 19 you know, considered them in -- as -- as I would 20 consider any -- any other evidence or opinion 21 on -- on these relevant subjects. 22 MS. BROWN: 23 Q The FDA went on to conclude, Doctor, 24 that, in light of the shortcoming, a panel of</p>
<p style="text-align: right;">Page 199</p> <p>1 specific determination. 2 But I was basing my opinions on the 3 general behavior, summarized behavior of talc 4 based on the available evidence. 5 Q In forming your opinions in this case, 6 Doctor, have you concluded that a particular 7 route of exposure is more likely when women are 8 using talcum powder products perineally? 9 MS. O'DELL: 10 Object to the form. 11 A Certainly it would seem logical that 12 the route of talc exposure would be related to 13 the area that the talc is used. 14 MS. BROWN: 15 Q As such, do you believe and have you 16 assumed for purposes in your -- of your opinions 17 in this case that talc more likely migrates from 18 the perineum to the ovaries, as opposed to talc 19 being inhaled and then traveling down to the 20 ovaries? 21 A The evidence I've seen would suggest 22 that that migration that you described from the 23 perineum through the vagina into the fallopian 24 tubes into the ovary is certainly far more likely</p>	<p style="text-align: right;">Page 201</p> <p>1 experts at the 1994 ISRTP/FDA workshop declared 2 that the 1993 NTP study has no relevance to human 3 risk. 4 Do you share that conclusion? 5 MS. O'DELL: 6 Object to the form. 7 A I do not. And I think, importantly, 8 you know, even there at the bottom of page 4, 9 their point number 4 saying a cogent biological 10 mechanism by which talc might lead to ovarian 11 cancer is lacking. 12 MS. BROWN: 13 Q Uh-huh. 14 A I believe, as we're discussing today, 15 subsequent research and subsequent studies 16 have -- and including my report, have helped 17 define that plausible biological mechanism 18 which -- by which talc may lead to ovarian 19 cancer. 20 Q In answering my question, Doctor, you 21 pointed to a different portion of the same page 22 we were discussing; correct? 23 A Correct. 24 Q And what you pointed to was the FDA's</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 202</p> <p>1 conclusion here in 2014 that a cogent biological</p> <p>2 mechanism by which talc might lead to ovarian</p> <p>3 cancer is lacking. Correct?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form.</p> <p>6 A I -- I would disagree in the general</p> <p>7 nature of your statement and clarify it by saying</p> <p>8 the FDA found a lack of that mechanism based on</p> <p>9 the submitted literature of the citizen petition.</p> <p>10 MS. BROWN:</p> <p>11 Q So do you understand, Doctor, in</p> <p>12 evaluating the FDA's response, that they, in</p> <p>13 fact, did their own investigation in addition to</p> <p>14 the literature that was provided to them at the</p> <p>15 time?</p> <p>16 MS. O'DELL:</p> <p>17 Objection. Misstates the record.</p> <p>18 A Well, my reading of it, it says</p> <p>19 they -- that their -- that the scientific</p> <p>20 literature considered was submitted in support of</p> <p>21 both citizen petitions. And...</p> <p>22 MS. BROWN:</p> <p>23 Q Are you finished, Doctor?</p> <p>24 A Yes. I was just looking to see if</p>	<p style="text-align: right;">Page 204</p> <p>1 A I -- I disagree with the -- or I -- I</p> <p>2 have found, based on a review of the literature,</p> <p>3 that there are now additional supporting studies</p> <p>4 that would -- that would refute some of these</p> <p>5 conclusions of -- by the FDA review.</p> <p>6 Q And explain to us, then, Doctor, what</p> <p>7 methodology you employed or what research you</p> <p>8 conducted to reach a conclusion different from</p> <p>9 the FDA's conclusion in 2014.</p> <p>10 A I think, similar to what the FDA</p> <p>11 described, my review is of the literature now,</p> <p>12 you know, through 2018, examining the available</p> <p>13 information regarding inflammatory response to</p> <p>14 talc and then talc exposure as it relates</p> <p>15 to -- to the initiation of progression of cancer.</p> <p>16 Q Dr. Leavy -- Dr. Levy, do you think</p> <p>17 that the FDA, in concluding, as they did in 2014,</p> <p>18 that a cogent biological mechanism by which talc</p> <p>19 might lead to ovarian cancer is lacking, do you</p> <p>20 think they were wrong at that time?</p> <p>21 A I would -- I -- I would say that they</p> <p>22 were incomplete at that time. And, in fact, you</p> <p>23 know, one of the --</p> <p>24 If we -- if we look at page 5 in the</p>
<p style="text-align: right;">Page 203</p> <p>1 there was a notation about further --</p> <p>2 Q I'll direct you, Doctor, to page 4, the</p> <p>3 second full paragraph that begins "In addition,</p> <p>4 the FDA stated."</p> <p>5 "In addition, we reviewed relevant</p> <p>6 toxicity literature (consisting of 15 articles</p> <p>7 from 1980 to 2008) not cited in your petition to</p> <p>8 determine if there was additional support at this</p> <p>9 point in time for your suggested warning label."</p> <p>10 Do you see that?</p> <p>11 A I do.</p> <p>12 Q And, based on the FDA's review of all</p> <p>13 the literature that they investigated at the</p> <p>14 time, they concluded that a cogent biological</p> <p>15 mechanism by which talc might lead to ovarian</p> <p>16 cancer was lacking. Right?</p> <p>17 MS. O'DELL:</p> <p>18 Objection to form.</p> <p>19 MS. BROWN:</p> <p>20 Q That was their conclusion; correct?</p> <p>21 A Yes, as written, that was their -- that</p> <p>22 was the FDA's conclusion.</p> <p>23 Q And you, Dr. Levy, disagree with that</p> <p>24 conclusion; correct?</p>	<p style="text-align: right;">Page 205</p> <p>1 one, two -- third full paragraph beginning with</p> <p>2 "while there exists," where the FDA does agree</p> <p>3 about the -- that it's plausible that perineal</p> <p>4 talc and other particulates reach the endometrial</p> <p>5 cavity and -- and associated organs and may</p> <p>6 elicit a foreign-body-type reaction and</p> <p>7 inflammatory response that in some exposed women</p> <p>8 may progress to epithelial cancers. What they do</p> <p>9 state, "However, there has been no conclusive</p> <p>10 evidence to support causality."</p> <p>11 So I would suggest that this paragraph</p> <p>12 is in support of the biologically plausible</p> <p>13 mechanism that I included in the report and</p> <p>14 that -- and, as we've been discussing, I</p> <p>15 haven't -- we -- we've not been discussing a</p> <p>16 causal or a formal causal evaluation.</p> <p>17 Q What information did you rely on,</p> <p>18 Doctor, in reaching the conclusion that there is</p> <p>19 a biological mechanism that the FDA did not?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form. Misstates his</p> <p>22 testimony.</p> <p>23 A I'm stating that the -- as we</p> <p>24 discussed, as we've been discussing today, the --</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 206</p> <p>1 the response to talc -- the response to talc</p> <p>2 exposure as an inflammatory response is supported</p> <p>3 by a number of studies, including the NTP study,</p> <p>4 which, although the FDA had some concerns with,</p> <p>5 the FDA also made statements regarding the</p> <p>6 exposure to talc and other particulates having an</p> <p>7 inflammatory response and that some exposed</p> <p>8 women's may have progressed to epithelial</p> <p>9 cancers.</p> <p>10 So, again, they're -- I think</p> <p>11 they -- they're in agreement there. So even the</p> <p>12 concerns with the study withstanding, there's --</p> <p>13 there's -- there's -- I still -- I still think</p> <p>14 the FDA report is in support of the mechanism</p> <p>15 that we've been discussing.</p> <p>16 MS. BROWN:</p> <p>17 Q The FDA concludes that a cogent</p> <p>18 biological mechanism by which talc might lead to</p> <p>19 ovarian cancer is lacking, do they not?</p> <p>20 MS. O'DELL:</p> <p>21 Objection to form. Asked and answered.</p> <p>22 A But I would al- -- I would say the FDA</p> <p>23 contr- -- perhaps contradicts itself later in the</p> <p>24 same document, stating that there is both an</p>	<p style="text-align: right;">Page 208</p> <p>1 studies on which you're relying?</p> <p>2 A Not -- not for the contents of the</p> <p>3 report. Not that I'm aware of. I think we've --</p> <p>4 we've already discussed some of the other</p> <p>5 references contained in the report</p> <p>6 below and -- or at least by mention and Gates.</p> <p>7 (DEPOSITION EXHIBIT NUMBER 17</p> <p>8 WAS MARKED FOR IDENTIFICATION.)</p> <p>9 MS. BROWN:</p> <p>10 Q I'm gonna mark as Exhibit 17 to your</p> <p>11 deposition the Buz'Zard study that you mentioned</p> <p>12 a moment ago. Do you recall that?</p> <p>13 A Yes.</p> <p>14 Q Do you rely on the Buz'Zard study in</p> <p>15 supporting your view that chronic inflammation</p> <p>16 from talcum powder use can cause ovarian cancer?</p> <p>17 MS. O'DELL:</p> <p>18 17?</p> <p>19 MS. BROWN:</p> <p>20 Yes.</p> <p>21 A Sorry. Can you restate your question?</p> <p>22 It wasn't...</p> <p>23 MS. BROWN:</p> <p>24 Q Do you rely on what we've marked as</p>
<p style="text-align: right;">Page 207</p> <p>1 inflammatory response and that in some exposed</p> <p>2 women they may progress to epithelial cancer.</p> <p>3 MS. BROWN:</p> <p>4 Q Other than the Woodruff article,</p> <p>5 Doctor, are you aware of any other study in</p> <p>6 animals that shows inflammation leading to</p> <p>7 cancer?</p> <p>8 MS. O'DELL:</p> <p>9 Objection to form. Other than those</p> <p>10 he's mentioned?</p> <p>11 A Yeah. I -- I would have to -- that</p> <p>12 would -- that would require review of the</p> <p>13 literature to -- to speak generally to animal</p> <p>14 studies and inflammation leading to cancer.</p> <p>15 MS. BROWN:</p> <p>16 Q Let me rephrase.</p> <p>17 In terms of your opinion here that talc</p> <p>18 causes chronic inflammation that causes ovarian</p> <p>19 cancer, you identified the Hamilton study, the</p> <p>20 NTP study, and the Woodruff study as animal</p> <p>21 studies that support that view. True?</p> <p>22 A I identified those studies as</p> <p>23 supportive of my -- of my opinion, yes.</p> <p>24 Q Are you aware of any additional animal</p>	<p style="text-align: right;">Page 209</p> <p>1 Exhibit 17, the Buz'Zard study, to support your</p> <p>2 view that talcum powder causes chronic</p> <p>3 inflammation that leads to ovarian cancer?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form.</p> <p>6 A As we've discussed, not singularly, but</p> <p>7 the -- as part -- as part of a complete picture</p> <p>8 of talc causing reactive oxygen species</p> <p>9 generation and other inflammatory responses,</p> <p>10 certainly this is a study that supports that</p> <p>11 opinion.</p> <p>12 MS. BROWN:</p> <p>13 Q Did you consider the type of cells that</p> <p>14 were evaluated in the Buz'Zard study?</p> <p>15 MS. O'DELL:</p> <p>16 Objection to form. Vague.</p> <p>17 A Certainly in terms of the overall</p> <p>18 experimental design.</p> <p>19 MS. BROWN:</p> <p>20 Q Did those -- were those normal human</p> <p>21 ovarian cells?</p> <p>22 A The -- the author has labeled them as</p> <p>23 normal human ovarian cells. But the -- you know,</p> <p>24 one of the key characteristics and similar to our</p>

Shawn Levy, Ph.D.

Page 210	Page 212
<p>1 comments on -- on animal systems is all -- all</p> <p>2 in vitro or in vivo studies that are using cell</p> <p>3 lines or animals have limitations. And in this</p> <p>4 case, you know, cell lines are particularly</p> <p>5 notorious in research in general for</p> <p>6 their -- for -- having to use care in extending</p> <p>7 findings to, you know, broad mechanisms in a --</p> <p>8 in a complex organism or in the human body.</p> <p>9 Q Sure.</p> <p>10 What you're -- what you're saying is</p> <p>11 you've got to be careful taking the findings from</p> <p>12 one cell study and extrapolating that to humans.</p> <p>13 Fair?</p> <p>14 MS. O'DELL:</p> <p>15 Object to the form.</p> <p>16 A The -- I think you have to be careful</p> <p>17 in evaluating each study in using the relevant</p> <p>18 components of that study and observations in that</p> <p>19 study as part of an overall mechanism and whether</p> <p>20 it's supportive or refutes such a mechanism.</p> <p>21 So --</p> <p>22 MS. BROWN:</p> <p>23 Q Did -- did you exercise that care here</p> <p>24 as it relates to the Buz'Zard study?</p>	<p>1 MS. O'DELL:</p> <p>2 Figure 3.</p> <p>3 A Figure 3?</p> <p>4 The one interesting observation in</p> <p>5 these two figures, both Figure 3A and Figure 3B,</p> <p>6 being the percentage of reactive oxygen specie</p> <p>7 generation in two different cell types, one in --</p> <p>8 one in Panel A and one in Panel B, is -- what I</p> <p>9 did not see included, if I --</p> <p>10 And I'm reading to see if I recall</p> <p>11 correctly.</p> <p>12 -- was a -- the -- the cell viability</p> <p>13 assay that they use for normalization has</p> <p>14 a -- somewhat of a limitation in that it -- it</p> <p>15 doesn't measure cell senescence. It only</p> <p>16 measures cell death. And, so, they -- not to</p> <p>17 dis- -- not that I disagree with your observation</p> <p>18 that it did not show the sig- -- significant</p> <p>19 increase, but there is the possibility that the</p> <p>20 reason that you see an actual decrease in the RS</p> <p>21 generation at the higher doses of talc is that</p> <p>22 cells have gone senescent and are essentially no</p> <p>23 longer responding to that increased dose.</p> <p>24 So I think there's at least two</p>
Page 211	Page 213
<p>1 A So the Buz'Zard study, you know,</p> <p>2 primarily, as -- as referenced, was to illustrate</p> <p>3 a study that showed an increase in reactive</p> <p>4 oxygen species generation, and that's the -- the</p> <p>5 primary purpose, or I should say primary</p> <p>6 observation on the -- from this.</p> <p>7 Now, certainly, the study contained</p> <p>8 more observations than that and certainly had</p> <p>9 some -- you know, a number of other components.</p> <p>10 Q How does the Buz'Zard study support</p> <p>11 your view that talcum powder causes chronic</p> <p>12 inflammation that causes ovarian cancer?</p> <p>13 A So the Buz'Zard study supports the view</p> <p>14 that exposure to talcum powder causes an</p> <p>15 inflammatory response.</p> <p>16 Q And that inflammatory response you saw</p> <p>17 in the Buz'Zard study does not increase with</p> <p>18 increasing doses of talcum powder. Correct?</p> <p>19 A I have to review. I believe that -- I</p> <p>20 believe their figures suggest --</p> <p>21 You know, are you referring</p> <p>22 specifically to their reaction -- reactive oxygen</p> <p>23 specie generation?</p> <p>24 Q Correct.</p>	<p>1 different ways to interpret some of these</p> <p>2 results. But I don't disagree with your</p> <p>3 observations regarding Figure 3.</p> <p>4 MS. BROWN:</p> <p>5 Q This study was conducted in a</p> <p>6 nutritional lab, not a cancer lab. True?</p> <p>7 A I'm -- I'm not aware of the type of</p> <p>8 laboratory or even the...</p> <p>9 Q And the study was -- the purpose of the</p> <p>10 study was to assess whether there was a certain</p> <p>11 effect of pine bark supplement? Is that right?</p> <p>12 MS. O'DELL:</p> <p>13 Objection to form.</p> <p>14 A They were looking at the -- the effect</p> <p>15 of a proprietary -- as stated by the authors, a</p> <p>16 proprietary mixture of water soluble</p> <p>17 bioflavonoids extracted from French maritime pine</p> <p>18 bark.</p> <p>19 MS. BROWN:</p> <p>20 Q Uh-huh.</p> <p>21 And did you investigate whether the</p> <p>22 ovarian cells that they used here were</p> <p>23 genetically altered?</p> <p>24 A No, I did not investigate that.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 214</p> <p>1 Q Did you --</p> <p>2 I'm sorry. Were you done?</p> <p>3 A No. I would say it's fair -- it's fair</p> <p>4 to say that, you know, that the -- whether</p> <p>5 they're genetically altered or not, the -- the --</p> <p>6 you know, the same potential limitations as far</p> <p>7 as extrapolation to the human system would apply</p> <p>8 for any signs.</p> <p>9 But, again, the purpose of the Buz'Zard</p> <p>10 study, as -- as referenced in the report, was to</p> <p>11 indicate that there are studies that have shown</p> <p>12 an increase in reactive oxygen specie generation</p> <p>13 under exposure to -- to talc. And I think the</p> <p>14 study is reasonably clear on that increase</p> <p>15 relative to control.</p> <p>16 Q Except what this study showed, Doctor,</p> <p>17 is the more talc you give, the decrease from</p> <p>18 baseline in the reactive oxygen species.</p> <p>19 Correct?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form. Asked and</p> <p>22 answered. Misstates the testimony.</p> <p>23 MS. BROWN:</p> <p>24 Q Take a look at Figure 3; right, Doctor?</p>	<p style="text-align: right;">Page 216</p> <p>1 Q My question was, Doctor, what this</p> <p>2 study shows is the more talc you give, the less</p> <p>3 ROS generation there is. True?</p> <p>4 MS. O'DELL:</p> <p>5 Objection to form.</p> <p>6 A Again, under -- under the conditions of</p> <p>7 this particular study.</p> <p>8 MS. BROWN:</p> <p>9 Q Do you think the Buz'Zard study is</p> <p>10 scientifically reliable?</p> <p>11 A I have no basis to -- to suggest that</p> <p>12 it's -- that it's not reliable.</p> <p>13 Q Do you think that --</p> <p>14 A But I think there -- it does -- if</p> <p>15 there is a -- as we discussed earlier, an</p> <p>16 importance to not overgeneralize conclusions or</p> <p>17 lack of conclusions as, you know, outside of the</p> <p>18 system under study.</p> <p>19 Q If -- I want you to assume that the</p> <p>20 Buz'Zard study used genetically altered ovarian</p> <p>21 cells that did not have the p53 protein. Would</p> <p>22 that affect your analysis of Buz'Zard?</p> <p>23 MS. O'DELL:</p> <p>24 Object to the form.</p>
<p style="text-align: right;">Page 215</p> <p>1 A No. I agree. But, as stated, and an</p> <p>2 important clarification is whether that decrease</p> <p>3 is significant relative to the biology is -- is</p> <p>4 unknown.</p> <p>5 Q Right.</p> <p>6 This study certainly does not</p> <p>7 conclusively show that the more talc you give,</p> <p>8 the more ROS is generated. Correct?</p> <p>9 MS. O'DELL:</p> <p>10 Object to the form.</p> <p>11 A In these particular cell lines under</p> <p>12 these conditions, the -- the study certainly did</p> <p>13 not draw that conclusion.</p> <p>14 MS. BROWN:</p> <p>15 Q In fact, what this study shows is the</p> <p>16 more talc you give, the less of -- of ROS</p> <p>17 generation you have. Doesn't it?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A I think importantly in this study, the</p> <p>21 time dependency for each of the doses is more</p> <p>22 important at the doses rather than comparing dose</p> <p>23 to dose.</p> <p>24 MS. BROWN:</p>	<p style="text-align: right;">Page 217</p> <p>1 A Well, that's -- that's an impossible</p> <p>2 question. Like you can't have --</p> <p>3 Well, you can't call a cell type a</p> <p>4 normal ovarian cell and -- absent p53 protein.</p> <p>5 You're -- it'd be -- you're fundamentally</p> <p>6 changing the biology of the cell as it relates to</p> <p>7 ovarian cancer or cancer in general.</p> <p>8 MS. BROWN:</p> <p>9 Q Because p53 is something that you have</p> <p>10 in your genes that prevents against ovarian</p> <p>11 cancer. True?</p> <p>12 MS. O'DELL:</p> <p>13 Objection.</p> <p>14 A So p5- -- p53 is a well-known, often</p> <p>15 mutated gene in a number of human cancers.</p> <p>16 MS. BROWN:</p> <p>17 Q And, so, if the ovarian cells that were</p> <p>18 studied in Buz'Zard did not have p53, it will</p> <p>19 call into question the study. Fair?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 A It would be difficult to answer. From</p> <p>23 the perspective of the presence or absence</p> <p>24 of -- of p53 having an effect on the ability of a</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 218</p> <p>1 cell to generate reactive oxygen species under --</p> <p>2 under exposure to a substance like talcum powder</p> <p>3 would need to be tested directly.</p> <p>4 MS. BROWN:</p> <p>5 Q Fair to say, in your mind, a cell</p> <p>6 missing p53 is not a normal human ovarian cell.</p> <p>7 True?</p> <p>8 A That is true.</p> <p>9 (DEPOSITION EXHIBIT NUMBER 18</p> <p>10 WAS MARKED FOR IDENTIFICATION.)</p> <p>11 MS. BROWN:</p> <p>12 Q Handing you what we've marked as</p> <p>13 Exhibit 18 to your deposition, it's a review</p> <p>14 article titled "Perineal Talc Use and Ovarian</p> <p>15 Cancer," by Ross Penninkilampi.</p> <p>16 Do you see that?</p> <p>17 A I do.</p> <p>18 Q This is an article that you cited in</p> <p>19 your report; correct?</p> <p>20 A Correct.</p> <p>21 Q Does this article support your view</p> <p>22 that there is a biolo -- in part --</p> <p>23 Strike that.</p> <p>24 Does this article, in part, support</p>	<p style="text-align: right;">Page 220</p> <p>1 available literature and, in this case, review a</p> <p>2 meta-analysis of some reasonably large-scale</p> <p>3 studies to try to bring the proposed biologically</p> <p>4 plausible mechanism and include the -- the</p> <p>5 available epidemiological information for those,</p> <p>6 such as the Penninkilampi and Eslick paper we're</p> <p>7 discussing.</p> <p>8 Q What methodology did you employ in</p> <p>9 terms of reviewing the Penninkilampi findings as</p> <p>10 it relates to the question you addressed in your</p> <p>11 report?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form.</p> <p>14 A I -- I used the same methodology for</p> <p>15 the other studies as a review of the paper and</p> <p>16 its -- and its methods and conclusions.</p> <p>17 MS. BROWN:</p> <p>18 Q Do you believe this review, systematic</p> <p>19 review and meta-analysis, provides evidence that</p> <p>20 there's a biologically plausible mechanism by</p> <p>21 which talc can cause ovarian cancer?</p> <p>22 A Yes. It provided -- it shows an</p> <p>23 association between talc use and ovarian cancer.</p> <p>24 I don't -- I don't believe this particular study</p>
<p style="text-align: right;">Page 219</p> <p>1 your opinion in this case that there is a</p> <p>2 biologically plausible mechanism by which talcum</p> <p>3 powder can cause ovarian cancer which can</p> <p>4 cause --</p> <p>5 Strike that. Gonna do it again.</p> <p>6 Does this article support your view, in</p> <p>7 part, that talcum powder can cause chronic</p> <p>8 inflammation that can cause ovarian cancer?</p> <p>9 A This is an article I considered in</p> <p>10 the -- in the overall review and, in the</p> <p>11 conclusions of this article, found a -- an</p> <p>12 association between perineal talc use and ovarian</p> <p>13 cancer, according to the authors.</p> <p>14 So it was supportive of the proposed</p> <p>15 mechanism but was, again, in part.</p> <p>16 Q And, on page 13 and 14 of your report,</p> <p>17 you, in fact, reference the Penninkilampi study</p> <p>18 and some of its conclusions; correct?</p> <p>19 A Correct. On the -- on the bottom of</p> <p>20 page 13, yes.</p> <p>21 Q And what was the purpose of including</p> <p>22 this description of Penninkilampi in your expert</p> <p>23 report, Doctor?</p> <p>24 A Just to be sure to be -- to include</p>	<p style="text-align: right;">Page 221</p> <p>1 goes on to specifically elucidate causation, but</p> <p>2 it certainly shows the association.</p> <p>3 Q Well, the study specifically says that</p> <p>4 causation cannot be found, based on the results.</p> <p>5 Right?</p> <p>6 MS. O'DELL:</p> <p>7 Objection to form.</p> <p>8 MS. BROWN:</p> <p>9 Q If you look at page 42, Doctor, the</p> <p>10 very end of that first paragraph, "A certain</p> <p>11 causal link between talc use and ovarian cancer</p> <p>12 has not been established."</p> <p>13 Do you see that?</p> <p>14 MS. O'DELL:</p> <p>15 Where are you? Page 42. Where are you</p> <p>16 reading, please?</p> <p>17 MS. BROWN:</p> <p>18 Page 42, the end of the first</p> <p>19 paragraph.</p> <p>20 A Yes, I see that.</p> <p>21 MS. BROWN:</p> <p>22 Q Do you agree with that statement,</p> <p>23 Doctor, that a causal link between talc use and</p> <p>24 ovarian cancer has not yet been established?</p>

56 (Pages 218 to 221)

Shawn Levy, Ph.D.

Page 222	Page 224
<p>1 MS. O'DELL: 2 Objection. 3 A No, I wouldn't. But, again, my review 4 of this was to tie the biologically plausible 5 mechanism to, you know, human observation, not 6 provide a evaluation of the -- of the causal 7 link. 8 And I think the -- I would suspect that 9 the -- 10 I'm also not aware of a study that has 11 been able to -- or a -- or a -- what would be 12 necessary -- 13 I'm not aware of a study that has been 14 able to provide all of the recognized and 15 established methodology for causation and have 16 that applied in -- in talc. 17 MS. BROWN: 18 Q You're not aware of any study in the 19 talc epidemiology that has concluded that talcum 20 powder causes ovarian cancer; correct? 21 MS. O'DELL: 22 Objection to form. 23 A I'm aware of a number of studies that 24 have shown a strong correlation between the two.</p>	<p>1 examine that comprehensively, when you consider 2 the etiology of a disease and the latency periods 3 that have been observed in ovarian cancer in 4 general and the meta review by both this earlier 5 paper by Penninkilampi and then their subsequent 6 later work, you have a challenge of a -- in a 7 cohort study, a disease that is somewhat rare, 8 coupled with a exposure and latency period that's 9 been, in the -- in the limited number of studies 10 that have looked at this, appears to be quite 11 long, and then when you couple in the -- the 12 ethical concerns of actually performing a trial, 13 where it becomes a very difficult causation bar 14 to reach. 15 And, so, instead, we rely on the 16 case -- the available case-control data and then 17 systematic and meta-analysis reviews such as some 18 of the epidemiologists have performed to make 19 assessments into the likelihood that -- and the 20 strength of the association between talc use and 21 ovarian cancer. 22 Q Are you intending to provide an opinion 23 on the strength of the association between talc 24 use and ovarian cancer as evidenced in the</p>
Page 223	Page 225
<p>1 But I would have to defer to the epidemiology 2 expert witnesses as to their opinion on 3 causation. 4 MS. BROWN: 5 Q One of the things you told us that you 6 reviewed in connection with your opinion was the 7 talc epidemiology. Is that right? 8 A That's right. 9 Q Did you conduct a review of all of the 10 available epidemiology on talcum powder use and 11 ovarian cancer? 12 A I certainly tried to review it as 13 comprehensively as -- as possible. 14 Q And, in connection with that review, 15 you'll agree there is not a single study that 16 concludes there is a causal association between 17 talcum powder use and ovarian cancer; correct? 18 MS. O'DELL: 19 Objection to form. 20 A So I would -- I would -- interestingly, 21 there -- it's -- it becomes a -- as more -- as 22 more and more information has become available 23 over the last few years, that becomes a more and 24 more difficult bar to meet, simply because, to</p>	<p>1 epidemiology? 2 MS. O'DELL: 3 Object to the form. 4 A No. My -- my opinions are limited to 5 the biologically plausible mechanism and then 6 examining whether that biologically plausible 7 mechanism presented is supported by observations 8 in -- in available human studies. 9 MS. BROWN: 10 Q And when you say your opinion is 11 limited to a biological plausible mechanism, are 12 you talking of the theoretical concept or are you 13 talking about in the context of women using 14 talcum powder perineally? 15 A In the context -- 16 MS. O'DELL: 17 Object to the form. 18 THE WITNESS: 19 Sorry. 20 MS. O'DELL: 21 Excuse me. 22 A In the -- in the context of women using 23 talcum powder perineally specifically, and 24 then -- and then certainly also the -- some of</p>

Shawn Levy, Ph.D.

Page 226	Page 228
<p>1 the fundamental aspects of that mechanism may</p> <p>2 apply to other exposures as well.</p> <p>3 MS. BROWN:</p> <p>4 Q Like what?</p> <p>5 A Well, the -- the other exposure we've</p> <p>6 been discussing, in -- in that some of the</p> <p>7 studies looked at inhalation exposure, et cetera.</p> <p>8 But the primary review and the primary</p> <p>9 opinion is based on the perineal use of talcum</p> <p>10 powder and that exposure that, as -- as we</p> <p>11 discussed earlier, has a -- certainly a strong</p> <p>12 association with perineal use and an exposure --</p> <p>13 exposure in the ovaries.</p> <p>14 Q Your opinion is that if a woman uses</p> <p>15 talcum powder perineally, there is a biologically</p> <p>16 plausible mechanism by which enough talcum powder</p> <p>17 can migrate from outside of her vagina to her</p> <p>18 ovary to cause chronic inflammation that can lead</p> <p>19 to ovarian cancer?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 A So I'd say that the first part of your</p> <p>23 question is well established and included in the</p> <p>24 statements from FDA and others that that</p>	<p>1 trial that would examine that in a well-powered</p> <p>2 fashion to answer that question directly. And,</p> <p>3 certainly, as of today, there would be some</p> <p>4 significant ethical concerns with that design.</p> <p>5 So, instead, we rely on the cohort and</p> <p>6 case-control studies that are available. And</p> <p>7 those, again, studies are supporting an</p> <p>8 association between talc use and ovarian cancer.</p> <p>9 MS. BROWN:</p> <p>10 Q Right. But I'm talking about for your</p> <p>11 opinion that it's biologically plausible for</p> <p>12 perineal use of talc to cause ovarian cancer,</p> <p>13 have you made a determination, in your mind, of</p> <p>14 how long that perineal use has to take place for?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 A I wasn't asked to provide -- to provide</p> <p>18 that opinion on -- and it -- on that length or</p> <p>19 exposure or duration.</p> <p>20 Again, it was -- the focus was on the</p> <p>21 biologically plausible mechanism that if you have</p> <p>22 a single exposure and that -- that that single</p> <p>23 exposure through to any other may be sufficient</p> <p>24 to trigger that mechanism.</p>
Page 227	Page 229
<p>1 migration does occur.</p> <p>2 And then the next step in the -- in the</p> <p>3 mechanism is that that causes inflammation which,</p> <p>4 again, as we've discussed, in a number of</p> <p>5 studies, that the inflammation occurs and then,</p> <p>6 in these human studies, in their systematic</p> <p>7 review, that there is a clear association or a --</p> <p>8 a observed association between perineal use of</p> <p>9 talc and the detection of ovarian cancer at some</p> <p>10 point in the -- in the women's lives and, in the</p> <p>11 case of the Penninkilampi, with a relationship to</p> <p>12 the number of lifetime applications.</p> <p>13 So considering those things together,</p> <p>14 yes, there is a biologically plausible mechanism</p> <p>15 for perineal talc use through to ovarian cancer.</p> <p>16 MS. BROWN:</p> <p>17 Q Have you -- is -- is your opinion that</p> <p>18 there's a biologically plausible mechanism</p> <p>19 dependent on a particular number of years of</p> <p>20 perineal use?</p> <p>21 MS. O'DELL:</p> <p>22 Objection to form.</p> <p>23 A The -- so the -- as we just discussed,</p> <p>24 there's no -- I can't point to a formal clinical</p>	<p>1 MS. BROWN:</p> <p>2 Q That's helpful, Doctor.</p> <p>3 So, as I understand your opinion, your</p> <p>4 piece of the puzzle here was to look at whether</p> <p>5 one single application of talcum powder to the</p> <p>6 perineum could lead to chronic inflammation that</p> <p>7 could cause ovarian cancer.</p> <p>8 MS. O'DELL:</p> <p>9 Objection.</p> <p>10 MS. BROWN:</p> <p>11 Q Correct?</p> <p>12 A No, no.</p> <p>13 MS. O'DELL:</p> <p>14 Object to the form of the question.</p> <p>15 A No. That's not my -- my statement.</p> <p>16 My statement was that, based on the</p> <p>17 evidence available, that there's a biologically</p> <p>18 plausible mechanism for the -- for the cellular</p> <p>19 changes that -- that is independent of the</p> <p>20 exposure.</p> <p>21 MS. BROWN:</p> <p>22 Q You've made a determin--</p> <p>23 A But certainly a single exposure would</p> <p>24 be the physically minimum number. And I</p>

58 (Pages 226 to 229)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 230</p> <p>1 believe -- I think we --</p> <p>2 Q That's what I want to understand. And</p> <p>3 how you -- how you make this biological</p> <p>4 plausibility determination is to evaluate a</p> <p>5 single exposure? Is that right?</p> <p>6 MS. O'DELL:</p> <p>7 Object to the form.</p> <p>8 A No.</p> <p>9 MS. O'DELL:</p> <p>10 Misstates his testimony.</p> <p>11 A That's -- that's not what I'm stating.</p> <p>12 My -- my statement is that the -- the</p> <p>13 biologically plausible mechanism is a mechanism</p> <p>14 that is independent of the exposure and that, as</p> <p>15 part of the description of that mechanism and the</p> <p>16 evaluation of the studies supporting that</p> <p>17 mechanism through an inflammatory response, the</p> <p>18 question of exposure, number, and duration,</p> <p>19 length of time, et cetera, would be a separate</p> <p>20 evaluation.</p> <p>21 MS. BROWN:</p> <p>22 Q Is your opinion that talcum powder</p> <p>23 products cause chronic inflammation that cause</p> <p>24 ovarian cancer limited to perineal use, or have</p>	<p style="text-align: right;">Page 232</p> <p>1 effect, it doesn't matter at all how much of the</p> <p>2 product is used?</p> <p>3 MS. O'DELL:</p> <p>4 Objection.</p> <p>5 MS. BROWN:</p> <p>6 Q Do you see what I'm struggling with?</p> <p>7 Can you help me understand? If I'm trying to</p> <p>8 figure out does X cause Y, it sounds like what</p> <p>9 you're saying is it doesn't matter how much X you</p> <p>10 have.</p> <p>11 MS. O'DELL:</p> <p>12 Objection to form.</p> <p>13 A So we're -- we're talking about</p> <p>14 mech- -- so mechanistic action --</p> <p>15 MS. BROWN:</p> <p>16 Q Okay.</p> <p>17 A -- which means the -- you set aside the</p> <p>18 "how much." And the question is, from -- on a</p> <p>19 molecular level, can the presence of a particular</p> <p>20 compound in a particular location cause a</p> <p>21 biological effect. And, so, that is the primary</p> <p>22 focus of the opinion in the -- in the paper or --</p> <p>23 sorry -- in my report.</p> <p>24 And then extending that to how much,</p>
<p style="text-align: right;">Page 231</p> <p>1 you also evaluated body use of talcum powder</p> <p>2 products?</p> <p>3 MS. O'DELL:</p> <p>4 Object to the form.</p> <p>5 A My -- my focus was on the perineal use,</p> <p>6 and that's where the majority of the studies</p> <p>7 have -- have examined. So the focus was on</p> <p>8 perineal use of talcum powder.</p> <p>9 MS. BROWN:</p> <p>10 Q And in conducting that evaluation, the</p> <p>11 results of which are contained in your report,</p> <p>12 you did not endeavor to quantify how much talcum</p> <p>13 powder used perineally could possibly migrate to</p> <p>14 the ovaries; is that right?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form. Asked and answered</p> <p>17 maybe ten times already today.</p> <p>18 But you may answer the question.</p> <p>19 A Yeah. I -- I wasn't asked to -- to</p> <p>20 provide that opinion or attempt that</p> <p>21 quantitation.</p> <p>22 MS. BROWN:</p> <p>23 Q So when you conduct your analysis of</p> <p>24 whether something can biologically cause an</p>	<p style="text-align: right;">Page 233</p> <p>1 how long, and the dur- -- and then the intensity</p> <p>2 or duration of the biological effect, again, is a</p> <p>3 separate -- would be a separate discussion or</p> <p>4 separate study.</p> <p>5 So, again, to clarify, the focus had</p> <p>6 been on that -- some of the fundamental</p> <p>7 mechanisms, talc -- a talcum powder exposure to</p> <p>8 an inflammatory response to the inflammatory</p> <p>9 response causing cancer.</p> <p>10 Again, the -- I would refer to and</p> <p>11 defer to the other experts in epidemiology</p> <p>12 regarding their opinions on the validity of</p> <p>13 the asso- -- validity and strength of the</p> <p>14 associations, again, from a formal epidemiology</p> <p>15 perspective.</p> <p>16 My review of those studies has ind- --</p> <p>17 has relied on their conclusions, and, then, in my</p> <p>18 own review of their -- of their methodology</p> <p>19 showing a increasing association, that is the</p> <p>20 bookends of my -- of the mechanism I proposed.</p> <p>21 So what this study is looking at is</p> <p>22 perineal use of talc, getting cancer.</p> <p>23 The -- what I've proposed is in the</p> <p>24 middle. But this, again, the epidemiology</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 234</p> <p>1 studies are asking how many times, what, and 2 where, but there's been no evaluation that I'm 3 aware of that looks at exactly how the talc was 4 applied, when and where. Instead, it was asked 5 number of lifetime applications, duration of use, 6 and examining latency period. 7 And when I examine that information 8 from the perspective of that biological 9 mechanism, I, you know, notice some parallels in 10 between latency period averaging roughly twenty 11 years, which -- which mimics somewhat what's 12 observed in the asbestos field as far as, you 13 know, lung effect latency. 14 And then that continues into the 15 constituent -- or the other constituent 16 components of some of the products, including 17 testing into asbestos and some of the -- and 18 heavy metal exposure, et cetera, that those are, 19 again, supportive and offer a potential 20 amplifying effect in that -- in that mechanism, 21 given the nature of those other components. 22 Q What's the scientific support for the 23 amplification effect you just described? 24 A Just that the presence of</p>	<p style="text-align: right;">Page 236</p> <p>1 that opinion from the observations of a couple of 2 different studies, including the recent Saed 3 paper that did look at the specific consumer 4 product every -- you know, showing a -- if we do 5 it by way of comparison, between the Buz'Zard 6 paper and the recent Saed, seemingly a larger 7 magnitude of reactive oxygen species generation. 8 But, again, that is a -- extrapolating against 9 two different studies. 10 Q Do you -- 11 MS. O'DELL: 12 Excuse me. We've been going about an 13 hour and 20 minutes, maybe a little more. 14 MS. BROWN: 15 I think a little less. But I'm gonna 16 finish up. Then we'll take a quick break. 17 Q Does that work for you, Doctor? 18 I just want to finish Penninkilampi if 19 we can. 20 MS. O'DELL: 21 How much more do you have to go? 22 MS. BROWN: 23 About five or ten minutes. 24 MS. O'DELL:</p>
<p style="text-align: right;">Page 235</p> <p>1 more -- the -- 2 So if we extend beyond the opinion that 3 talc, as a com- -- as a singular compound, causes 4 inflammation and then also, based on the reviewed 5 expert reports, find that testing of talc has 6 been shown to contain asbestos or asbestos 7 fibers, that the presence of now two potential 8 insulting -- 9 I'm making a hypothesis or making a 10 statement that the -- you can have -- the more 11 biologically active compounds you have in an 12 exposure such as talc plus asbestos plus chromium 13 and then plus a milieu of chemicals that are in 14 fragrances may have an amplification effect on 15 that exposure and as part of that overall 16 biological mechanism. 17 Q Are you relying on a particular article 18 or any published scientific support for the 19 amplification argument? 20 MS. O'DELL: 21 Object to the form. He's answered the 22 question. 23 A No. I -- I don't know of a study that 24 is delineated. The -- it would be synthesizing</p>	<p style="text-align: right;">Page 237</p> <p>1 If you need a break, we can break now. 2 Or we can keep -- if you would like to wait five 3 or ten minutes, that's fine. Whatever's best for 4 you, Doctor. 5 THE WITNESS: 6 Yeah, if we could break now, that would 7 be great. 8 VIDEOGRAPHER: 9 Going off the record. The time is 10 2:10 p.m. 11 (OFF THE RECORD.) 12 VIDEOGRAPHER: 13 We're back on the record. The time is 14 2:26 p.m. 15 MS. BROWN: 16 Q Welcome back, Doctor. 17 Before we took a break, we were 18 discussing the Penninkilampi article. Do you 19 remember that? 20 A I do. 21 Q And one of the things the authors of 22 this very recent meta-analysis discussed is the 23 potential mechanism of ovarian cancer. Correct? 24 And I'll direct your attention to the</p>

60 (Pages 234 to 237)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 238</p> <p>1 discussion that begins on page 45. In the second</p> <p>2 sentence, the authors conclude here that the</p> <p>3 mechanism by which perineal talc use may increase</p> <p>4 the risk of ovarian cancer is uncertain.</p> <p>5 Do you see that?</p> <p>6 A I see that sentence, yes.</p> <p>7 Q And they go on to discuss the theory</p> <p>8 that talc could produce a chronic inflammatory</p> <p>9 response which could predispose to the</p> <p>10 development of ovarian cancer.</p> <p>11 Do you see that?</p> <p>12 A Yes.</p> <p>13 Q Okay. And they go on to explain a</p> <p>14 little bit more about the theory. Do you see</p> <p>15 that?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A Specifically the sentence beginning</p> <p>19 with "it is argued"?</p> <p>20 MS. BROWN:</p> <p>21 Q Uh-huh. "It is argued that cellular</p> <p>22 injury, oxidative stress, and local increase in</p> <p>23 inflammatory mediators such as cytokines,</p> <p>24 prostaglandins may be mutagenic and, hence,</p>	<p style="text-align: right;">Page 240</p> <p>1 presence of the talc or a continued chronic</p> <p>2 immune response or chronic inflammatory response,</p> <p>3 again, either directly or indirectly related to</p> <p>4 the exposure, would help support a environment</p> <p>5 that would allow the cancer progression to occur.</p> <p>6 So that is simply delineating those --</p> <p>7 those two things as it relates to inflammation</p> <p>8 and talc exposure.</p> <p>9 Q So you described two potential</p> <p>10 responses to talc right now. Correct?</p> <p>11 MS. O'DELL:</p> <p>12 Objection to form.</p> <p>13 A At least two, yes.</p> <p>14 MS. BROWN:</p> <p>15 Q Okay. And one is an acute inflammatory</p> <p>16 response; correct?</p> <p>17 A Yes.</p> <p>18 Q And for that you point to the Saed data</p> <p>19 on reactive oxygen species; is that right?</p> <p>20 MS. O'DELL:</p> <p>21 Objection to form.</p> <p>22 A That is one example, yes.</p> <p>23 MS. BROWN:</p> <p>24 Q Okay. Are there -- is there other</p>
<p style="text-align: right;">Page 239</p> <p>1 promote carcinogenesis."</p> <p>2 Do you see that?</p> <p>3 A I see that.</p> <p>4 Q This sentence refers to chronic</p> <p>5 inflammation promoting cancer. Correct?</p> <p>6 MS. O'DELL:</p> <p>7 Object to the form.</p> <p>8 A No. This -- this refers to that the</p> <p>9 presence of -- proposed that talc as a</p> <p>10 foreign -- that the presence of a foreign body</p> <p>11 would instigate a chronic inflammatory response.</p> <p>12 That's the statement in the paper.</p> <p>13 MS. BROWN:</p> <p>14 Q Is it your opinion that talcum powder</p> <p>15 can cause chronic inflammation that initiates</p> <p>16 cancer?</p> <p>17 A It's -- so it is -- it is my opinion</p> <p>18 is, part of the mechanism, that talcum powder can</p> <p>19 have two effects related to inflammation. The</p> <p>20 first effect is an acute effect resulting in</p> <p>21 cellular damage, and that is supported by the</p> <p>22 study showing increase in reactive oxygen species</p> <p>23 related to talc.</p> <p>24 The -- beyond that, the continued</p>	<p style="text-align: right;">Page 241</p> <p>1 scientific support for your opinion that talc can</p> <p>2 cause acute inflammation?</p> <p>3 A So it's any of the similar studies to</p> <p>4 Saed. And I would have to double-check the</p> <p>5 references, but they would have -- you know, any</p> <p>6 of the --</p> <p>7 MS. O'DELL:</p> <p>8 Feel free to --</p> <p>9 MS. BROWN:</p> <p>10 Q Buz'Zard?</p> <p>11 A So Buz'Zard would be one. Harper and</p> <p>12 Saed is -- is another.</p> <p>13 Q In your --</p> <p>14 A And so -- yeah. Yes, Buz'Zard and Lau</p> <p>15 and then -- yeah. So that would --</p> <p>16 Q Okay. So for your opinion that talc</p> <p>17 causes an acute inflamm- -- inflammatory</p> <p>18 response, you rely on the cell studies done by</p> <p>19 Saed and Buz'Zard; correct?</p> <p>20 MS. O'DELL:</p> <p>21 Objection to the form.</p> <p>22 A Yes, among others.</p> <p>23 MS. BROWN:</p> <p>24 Q In your opinion, Doctor, does that</p>

61 (Pages 238 to 241)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 242</p> <p>1 acute inflammatory response resolve? 2 A I don't -- I don't have any evidence to 3 suggest it resolves or not. The -- 4 Again, getting back to the mechanism 5 that has been -- that I've described and is 6 supported by the literature we've been discussing 7 is that there is a acute response as well as 8 evidence for talc causing a more chronic 9 inflammatory response. And so I've proposed a 10 mechanism by which both of those can contribute 11 to or enhance the development of cancer. 12 Q Can both of those inflammatory 13 responses that you just described initiate 14 cancer? 15 MS. O'DELL: 16 Object to the form. Asked and 17 answered. 18 A They are certainly a component of that. 19 And so, again, to restate the 20 mechanism, the acute inflammatory response or 21 the -- the formation of reactive oxygen species 22 has been known for decades to cause cellular 23 damage, and then cellular damage can result in 24 mutation of -- of DNA.</p>	<p style="text-align: right;">Page 244</p> <p>1 they're not -- I don't have evidence to -- to 2 delineate those specifically, other than -- other 3 than the supported mechanism that an acute 4 response can cause cellular damage, and then a 5 chronic response can cause cellular damage and be 6 supportive of that continued -- that continued 7 transformation. 8 So they are -- they -- those -- those 9 two delineated immune responses can either work 10 in -- in concert with each other, but there is no 11 evidence to suggest that one is insufficient 12 relative to the other in terms of progression of 13 the disease. 14 And I think specific to the -- to the 15 supported mechanism is that there -- I'm not 16 making that distinction in the -- in the report. 17 MS. BROWN: 18 Q Right. In your report, you don't talk 19 about acute versus chronic inflammation. 20 Correct? 21 A That's correct. I don't delineate the 22 two. Right. 23 Q But, here today, as we discuss in more 24 detail your opinions, you're explaining that</p>
<p style="text-align: right;">Page 243</p> <p>1 And then when you also consider the 2 full constituents of the products, the potential 3 presence -- 4 And this gets back to our earlier 5 discussions about amplification. 6 Components such as chromium, which have 7 a direct DNA-damaging effect, can also 8 ampli- -- again, add to the level of cellular 9 damage present. 10 And then the continued inflammatory 11 response, whether it is a -- related to the 12 initial acute response and a continuation of that 13 or is a separate chronic inflammatory response 14 would then support the environment necessary for 15 the malignant transformation or the malignancy of 16 the cancer to become what we -- what we would 17 generally refer to as ovarian cancer. 18 Q In your opinion, the chronic 19 inflammation promotes the cancer but does not 20 initiate it? 21 MS. O'DELL: 22 Object to the form. Asked and 23 answered. 24 A No. So I wouldn't -- I would say</p>	<p style="text-align: right;">Page 245</p> <p>1 you're -- in your mind, you see two potential 2 inflammatory responses from talc. Right? 3 MS. O'DELL: 4 Object to the form. 5 A I would disagree. I would say that 6 I -- I -- based on the information and studies, 7 the -- the review of other expert reports, that 8 it presents a supported opinion that talc has an 9 ability to cause an acute response as well as a 10 chronic response. 11 And, so, then, today we are discussing 12 using that data in support of the -- of the 13 mechanism as to how those -- those two responses 14 can work together or separately in the 15 progression of ovarian cancer. 16 MS. BROWN: 17 Q At the time you wrote your report in 18 November of 2018, were you of the view that talc 19 can cause both acute and chronic inflammatory 20 response? 21 A Yes. I mean, it was -- I was of the 22 view it causes an inflammatory response. And 23 then, as I continued to review information 24 available, it became clear that the talc</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 246</p> <p>1 response, being an inflammatory response in 2 totality, may have the ability to have 3 those -- to -- to have two independent responses 4 in tissues. 5 Q And, in your opinion, can both the 6 acute inflammatory response and the chronic 7 inflammatory response separately cause ovarian 8 cancer? 9 A Under the -- the mechanism I've 10 proposed, yes, that would be a -- a possibility 11 that they could separately cause, given that 12 they -- they're both inflammatory responses, they 13 both cause cellular damage. 14 And in the case -- in this case, 15 delineating the acute from chronic was more to 16 clarify the cellular damage aspect, the 17 transformative aspect of cancer from the -- the 18 necessary tumor progression aspects of cancer to 19 actually progress to disease. 20 Q In your opinion, Doctor, does talc 21 always first cause an acute reaction and then a 22 chronic reaction? 23 MS. O'DELL: 24 Object to the form.</p>	<p style="text-align: right;">Page 248</p> <p>1 important. 2 Q So there is a length of time or an 3 amount of exposure that would cause a chronic 4 inflammation that is different from the length of 5 time and the magnitude of exposure that will 6 cause an acute inflammation? 7 MS. O'DELL: 8 Object to the form. Misstates his 9 testimony. 10 A Yeah, no. Not -- that's not what 11 I -- that's not what I've stated. 12 I've simply stated that if we -- if we 13 look at the -- what is known about inflammation 14 and the biological response to foreign bodies, 15 you can have an initial acute response mediated 16 by the immune system and mediated by some of the 17 cellular damage that takes place, and then that 18 same response may continue in a chronic form for 19 some period of time and at some level of 20 magnitude. 21 Now, certainly there is likely a 22 dependency or, I should say, likely a 23 relationship to the amount of exposure and the 24 magnitude of that response.</p>
<p style="text-align: right;">Page 247</p> <p>1 A I -- I -- I don't have evidence 2 to -- to state that and would defer to some of 3 the other expert witnesses, like Dr. Saed, for 4 opinions on acute response versus chronic. 5 MS. BROWN: 6 Q In your opinion, though, you have at 7 least delineated in your mind two different types 8 of inflammatory responses. Correct? 9 MS. O'DELL: 10 Objection to form. 11 A I've -- I have described two mechanisms 12 for inflammation that -- that both can -- are 13 both supportive of the overall mechanism that 14 we're discussing. 15 MS. BROWN: 16 Q And is it -- is there a length of time 17 that differentiates an acute inflammatory 18 response from a chronic inflammatory response? 19 A Certainly I would say there -- in my 20 opinion, there would -- it would be a potential 21 time dependency or a magnitude dependency to 22 delineate an acute versus chronic response. But, 23 again, for the purpose of the biological 24 mechanism, separating them on those lines is not</p>	<p style="text-align: right;">Page 249</p> <p>1 But, again, the -- the opinions here 2 are specific to the mechanism and the initial 3 elucidation of that response and, you know, 4 not -- not on a quantitation of a -- a 5 dose-response relation -- or a dose-response 6 curve or relationship. 7 MS. BROWN: 8 Q Do you believe that every time a talc 9 particle enters the human body, it produces a 10 inflammatory response? 11 A All of the evidence would suggest yes. 12 Q Have you considered Heller's 1996 study 13 on that score? 14 A I would have to -- 15 On the score of inflammatory response? 16 Q Do you recall that Heller looked at 17 benign ovarian tissue and identified the 18 potential presence of talc? 19 A Sounds familiar. 20 Q I'll hand it to you. 21 (DEPOSITION EXHIBIT NUMBER 19 22 WAS MARKED FOR IDENTIFICATION.) 23 MS. BROWN: 24 Q Handing you, Doctor, what we've marked</p>

Shawn Levy, Ph.D.

Page 250	Page 252
<p>1 Heller's '96 article as Exhibit 19.</p> <p>2 And what I want to ask you about is</p> <p>3 Heller's finding as it relates to no reaction to</p> <p>4 the talc particle. Did you consider that --</p> <p>5 MS. O'DELL:</p> <p>6 Object to the form.</p> <p>7 MS. BROWN:</p> <p>8 Q -- in forming your opinion here?</p> <p>9 MS. O'DELL:</p> <p>10 Excuse me. Object to the form.</p> <p>11 MS. BROWN:</p> <p>12 Q I'll direct you, Doctor.</p> <p>13 On page 1508 of the Heller article,</p> <p>14 right above the comments section, "The</p> <p>15 investigators on this study concluded no evidence</p> <p>16 or response to talc, such as foreign body giant</p> <p>17 cell reactions or fibrosis in the tissue."</p> <p>18 My question is whether, in your</p> <p>19 opinion, every time talc is -- enters the body,</p> <p>20 it necessarily produces an inflammatory response.</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A No. My opinion is that every time talc</p> <p>24 enters the body, that has the potential to cause</p>	<p>1 mechanism that talc causes inflammation and then</p> <p>2 inflammation has a role in ovarian cancer.</p> <p>3 Extending that to circumstances where</p> <p>4 an exposure would not cause inflammation is -- is</p> <p>5 not germane to that -- to that mechanism and, in</p> <p>6 fact, again, not supported by literature to show</p> <p>7 that, you know, that a single exposure or some</p> <p>8 number of exposures are necessary or sufficient</p> <p>9 for a particular phenotype.</p> <p>10 MS. BROWN:</p> <p>11 Q So this Heller study purports to have</p> <p>12 found talc in ovarian tissue without an</p> <p>13 inflammatory response; right?</p> <p>14 MS. O'DELL:</p> <p>15 Object to the form.</p> <p>16 A In looking at their --</p> <p>17 Just one moment.</p> <p>18 So this was a --</p> <p>19 So is your -- is your question that</p> <p>20 the -- if the -- if the author showed talc being</p> <p>21 present in normal ovarian tissue?</p> <p>22 Q Well, first my question is did you</p> <p>23 consider this article in connection with your</p> <p>24 opinions in the case?</p>
Page 251	Page 253
<p>1 an immune response.</p> <p>2 MS. BROWN:</p> <p>3 Q Have you made a determination about</p> <p>4 whether or not that always happens?</p> <p>5 A I'll have --</p> <p>6 MS. O'DELL:</p> <p>7 Object to the form. It's vague.</p> <p>8 A I'm not aware of any --</p> <p>9 There -- there -- these -- none of the</p> <p>10 studies that have been reviewed have been</p> <p>11 designed to answer the question of "if ever."</p> <p>12 MS. BROWN:</p> <p>13 Q So, in your view, then, it's an open</p> <p>14 question about whether talc can be inside the</p> <p>15 body and not produce an inflammatory response.</p> <p>16 MS. O'DELL:</p> <p>17 Object.</p> <p>18 MS. BROWN:</p> <p>19 Q Is that fair?</p> <p>20 MS. O'DELL:</p> <p>21 Excuse me. Objection to form.</p> <p>22 Misstates his testimony.</p> <p>23 A So my -- my -- my testimony regarding</p> <p>24 the mechanism is that there is a well-supported</p>	<p>1 A I don't recall this article</p> <p>2 specifically, and I don't believe I cited it.</p> <p>3 I guess there's -- no.</p> <p>4 Q And then my second question, Doctor, is</p> <p>5 is it your opinion that every time the human body</p> <p>6 is exposed to particles of talc, it necessarily</p> <p>7 produces an inflammatory response that can either</p> <p>8 promote or initiate cancer of the ovaries?</p> <p>9 MS. O'DELL:</p> <p>10 Object to the form.</p> <p>11 A No. My --</p> <p>12 MS. O'DELL:</p> <p>13 Vague.</p> <p>14 A My comment was that the -- that any</p> <p>15 exposure to talc, particularly the perineal</p> <p>16 exposure to talc, has the potential to cause an</p> <p>17 inflammatory reaction.</p> <p>18 I don't have any evidence that all of</p> <p>19 the studies that we've been reviewing are in</p> <p>20 support -- are in support of that mechanism, but</p> <p>21 I don't know of a study that perhaps has been</p> <p>22 able to draw a conclusion, from a similar size</p> <p>23 study, to show that you can get significant talc</p> <p>24 accumulation without an inflammatory response.</p>

64 (Pages 250 to 253)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 254</p> <p>1 MS. BROWN: 2 Q Do you think you need significant talc 3 accumulation in the human body to cause or 4 promote ovarian cancer? 5 MS. O'DELL: 6 Objection to form. 7 A I wasn't asked to -- to provide -- 8 provide that opinion. 9 And, again, referring to the studies 10 that have -- that were reviewed and included in 11 the report, there is a relationship between 12 lifetime exposure and an increased risk in the 13 epidemiology reports. 14 But more detail on that in this 15 discussion, I would defer to the epidemiology 16 experts. But the -- there -- there does appear 17 to be a -- more of a response based on more talc 18 in the -- in the studies referenced. 19 MS. BROWN: 20 Q So on -- 21 Do you have any reason to dispute the 22 findings of Heller here of talc in the ovaries 23 without a foreign body reaction? 24 MS. O'DELL:</p>	<p style="text-align: right;">Page 256</p> <p>1 A In -- in terms of cancer, the 2 epidemiology would suggest -- or I would say 3 the -- the evidence in the literature is -- does 4 not allow that question to be answered, and the 5 reason being is when you look at the latency of 6 the disease and the progression of the disease 7 and the challenges in detecting it, there just 8 has not been enough time with the, perhaps, rigor 9 of analysis that is undergoing now to make that 10 assessment of is it 100 percent of the time or is 11 it something less than 100 percent of the time. 12 I think, statistically speaking, 13 there -- the only data that -- that is available 14 for review is -- is what is contained in some of 15 the meta-analysis and epidemiology studies 16 showing a significant increased risk to ovarian 17 cancer based on exposure to talc. And it 18 would -- it would only be -- I think it would be 19 inappropriate at this time to try to infer what 20 percentage of time that would be indicative of 21 for exposure. 22 Q Have the plaintiffs' lawyers shared 23 with you expert reports from their expert 24 pathologists who have looked at ovarian tissue of</p>
<p style="text-align: right;">Page 255</p> <p>1 Objection. 2 A I guess my -- I have some -- I guess I 3 have some concerns with some of the methodology 4 as it relates to the detection of the... 5 MS. BROWN: 6 Q Do you think it's possible, Doctor, for 7 talc to enter the body and -- and be completely 8 inert and not cause any reaction? 9 MS. O'DELL: 10 Object to the form. 11 A So my -- the -- the mechanism I've 12 proposed is -- is based -- you know, based on the 13 literature, is that talc causes an inflammatory 14 response and that inflammatory response is 15 supportive of progression to ovarian cancer. 16 MS. BROWN: 17 Q Does that happen 100 percent of the 18 time? 19 MS. O'DELL: 20 Object to the form. In terms of 21 inflammatory response or in terms of cancer? 22 MS. BROWN: 23 Q If you don't understand the question, 24 you'll let me know.</p>	<p style="text-align: right;">Page 257</p> <p>1 plaintiffs in this litigation, purported to find 2 talc with no foreign body reaction? 3 MS. O'DELL: 4 Objection. There have been no 5 case-specific pathology reports disclosed in the 6 litigation we're here about today. And if 7 there's something else you're talking about, you 8 should be specific. 9 A The -- I don't recall a pathology 10 report. I've seen expert reports from 11 epidemiologists, OB-GYN and -- and some -- and 12 other scientists. But I don't recall a specific 13 pathology report. 14 MS. BROWN: 15 Q If the biologically plausible mechanism 16 that you posit in your report is true, would you 17 expect that the pathology slides of women with 18 ovarian cancer who have used talc would evidence 19 talcum powder with a foreign body reaction? 20 MS. O'DELL: 21 Object to the form. Incomplete 22 hypothetical. 23 A That, I would have to ask how you're 24 defining a foreign body reaction.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 258</p> <p>1 MS. BROWN:</p> <p>2 Q Well, would you expect to see some</p> <p>3 evidence of inflammation in the ovarian tissue of</p> <p>4 women who used talcum powder products?</p> <p>5 MS. O'DELL:</p> <p>6 Object to the form. Incomplete</p> <p>7 hypothetical.</p> <p>8 A Overall, speaking to, as we were</p> <p>9 discussing earlier, the potential for that</p> <p>10 inflammatory response remains. But given the</p> <p>11 heterogeneity in individuals, their overall</p> <p>12 health, their natural variation in the levels of</p> <p>13 activities of antioxidants, et cetera, I -- I</p> <p>14 would state that I would expect a variety of</p> <p>15 magnitude of response to a foreign body like talc</p> <p>16 among the individuals exposed to it.</p> <p>17 MS. BROWN:</p> <p>18 Q You'd expect to see something; right?</p> <p>19 MS. O'DELL:</p> <p>20 Object to the form.</p> <p>21 A No, not necessarily, because it -- it</p> <p>22 very much depends on the timing that's -- that is</p> <p>23 observed, how -- what methodology is used to</p> <p>24 detect the presence of talc or detect the</p>	<p style="text-align: right;">Page 260</p> <p>1 mentioned some of the other subtypes and the</p> <p>2 common gene mutations that go along with them and</p> <p>3 as, again, supportive of the same mechanism. And</p> <p>4 I think, if anything, the -- the current data</p> <p>5 would suggest a -- a higher prevalence of a</p> <p>6 particular subtype of cancer but certainly not</p> <p>7 the -- the mechanism doesn't -- is not exclusive</p> <p>8 to any one type.</p> <p>9 Q In your view, all types of epithelial</p> <p>10 ovarian cancer can be caused by inflammation?</p> <p>11 A No. That's -- that's not my statement.</p> <p>12 I would say all types of ovarian cancer are</p> <p>13 supported by an inflammatory response but that,</p> <p>14 as from a causative perspective, that's not what</p> <p>15 the mechanism is provided as an opinion as to</p> <p>16 cause. It's more that the -- an inflammatory</p> <p>17 response plays a role in disease initiation</p> <p>18 and/or progression.</p> <p>19 Q In your view, Dr. Levy, it is</p> <p>20 biologically plausible for inflammation to cause</p> <p>21 all types of epithelial ovarian cancer; true?</p> <p>22 A Again, I'm not -- I've not been</p> <p>23 speaking to inflammation as a causative -- as a</p> <p>24 cause of ovarian cancer. It is a factor in --</p>
<p style="text-align: right;">Page 259</p> <p>1 presence of the inflammatory response, if it's,</p> <p>2 you know, done histopathologically, if it is</p> <p>3 based on a reactive oxygen species assay.</p> <p>4 So given the -- speaking in general</p> <p>5 terms, I think it's just inappropriate to make a</p> <p>6 conclusion as to that, yes, you would always</p> <p>7 expect to see something.</p> <p>8 I would -- again, to restate what was</p> <p>9 stated earlier, any -- any exposure has the</p> <p>10 potential to cause that inflammatory response,</p> <p>11 and then the time, scale, and magnitude of that</p> <p>12 response is going to vary by person. Therefore,</p> <p>13 I would expect there would be a variability in</p> <p>14 individuals exposed to talc.</p> <p>15 MS. BROWN:</p> <p>16 Q Uh-huh. Is your opinion related to all</p> <p>17 the different histologic types of epithelial</p> <p>18 ovarian cancer?</p> <p>19 A My -- my opinion is not exclusive to</p> <p>20 any -- any one type. Certainly, the epithelial</p> <p>21 serous being the more common and most virulent</p> <p>22 type of cancers I think represents the most</p> <p>23 common.</p> <p>24 From a mechanistic perspective, I</p>	<p style="text-align: right;">Page 261</p> <p>1 in -- in disease progression.</p> <p>2 Q So when you conclude, as you do in your</p> <p>3 report, that talcum powder products cause chronic</p> <p>4 inflammation, you do not conclude that that</p> <p>5 chronic inflammation causes ovarian cancer?</p> <p>6 MS. O'DELL:</p> <p>7 Object to the form.</p> <p>8 A I wasn't asked to provide a causation.</p> <p>9 MS. BROWN:</p> <p>10 Q Your opinion here is limited to the</p> <p>11 potential for talcum powder products to produce</p> <p>12 inflammation; correct?</p> <p>13 MS. O'DELL:</p> <p>14 Object to the form.</p> <p>15 A No. My -- so my opinion is a -- is a</p> <p>16 supported plausible biological mechanism by which</p> <p>17 the exposure to talc can lead to ovarian cancer.</p> <p>18 And, in my opinion, as supported in the -- in the</p> <p>19 report, that is through an inflammatory response.</p> <p>20 MS. BROWN:</p> <p>21 Q I must be missing you, Doctor. So are</p> <p>22 you of the opinion that inflammation can cause</p> <p>23 ovarian cancer?</p> <p>24 A I'm of the opinion that inflammation is</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 262</p> <p>1 a component of ovarian cancer.</p> <p>2 Q Well, I'm not sure what you mean by</p> <p>3 that. Can inflammation cause ovarian cancer?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form. Asked and</p> <p>6 answered.</p> <p>7 A I'm asked -- I suppose -- again, the</p> <p>8 opinion here is of a mechanistic opinion, not a</p> <p>9 causation. I would defer to some of the</p> <p>10 epidemiology experts to have opinions on</p> <p>11 causation.</p> <p>12 MS. BROWN:</p> <p>13 Q You don't have an opinion on whether or</p> <p>14 not inflammation can cause ovarian cancer?</p> <p>15 MS. O'DELL:</p> <p>16 Different question.</p> <p>17 A Correct. That's a --</p> <p>18 As we've been discussing, my opinions</p> <p>19 are that inflammation is a component of ovarian</p> <p>20 cancer and can be attributed to aspects, not</p> <p>21 exclusively, but contributing to aspects of its</p> <p>22 initiation and aspects of its progression. But I</p> <p>23 did not say that ovarian cancer is caused by</p> <p>24 inflammation.</p>	<p style="text-align: right;">Page 264</p> <p>1 Well, first, we're -- I want to be</p> <p>2 cautious with our use of the word "cause"</p> <p>3 and -- because that's, as we've been discussing,</p> <p>4 this is a -- it is -- it is not controversial</p> <p>5 that ovarian cancer -- inflammation plays a role</p> <p>6 in ovarian cancer and -- and, again, my opinion</p> <p>7 is not towards causation.</p> <p>8 MS. BROWN:</p> <p>9 Q Well, I mean, tumors themselves elicit</p> <p>10 inflammatory responses; right?</p> <p>11 A What -- so what -- specifically, what</p> <p>12 are you referring to?</p> <p>13 Q Well, you talk about tumor-activated</p> <p>14 macrophages in your report; right?</p> <p>15 A Yes.</p> <p>16 Q There is an inflammatory response</p> <p>17 that's produced by the tumor itself; correct?</p> <p>18 A Yes. There are -- there -- there --</p> <p>19 there are absolutely cancer progression markers</p> <p>20 that are associated with continued inflammation.</p> <p>21 Q And that has nothing to do necessarily</p> <p>22 with the events that cause the cancer. Right?</p> <p>23 MS. O'DELL:</p> <p>24 Object to the form.</p>
<p style="text-align: right;">Page 263</p> <p>1 MS. BROWN:</p> <p>2 Q And what scientific support do you have</p> <p>3 for your opinion that inflammation is a component</p> <p>4 of ovarian cancer and can be attributed to</p> <p>5 aspects of ovarian cancer, including its</p> <p>6 initiation?</p> <p>7 A So, again, the synthesis of the -- of</p> <p>8 the papers we've been discussing, including Saed</p> <p>9 and others, showing the reactive oxygen species</p> <p>10 produced from talc. And, then, as far as</p> <p>11 inflammation and its role in cancer, there</p> <p>12 are -- and it's a fundamentally accepted aspect</p> <p>13 of cancer biology that's been around for -- for</p> <p>14 quite some time. And we mentioned earlier that</p> <p>15 there's a variety of review articles, including</p> <p>16 the ones we were comparing sentences to earlier</p> <p>17 today, that describe that in great detail.</p> <p>18 Q It's not generally accepted, though,</p> <p>19 that ovarian cancer is caused by inflammation.</p> <p>20 Fair?</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A I think there's a number of studies</p> <p>24 that --</p>	<p style="text-align: right;">Page 265</p> <p>1 A Well, so the -- we -- we would be going</p> <p>2 down a slightly different road. And if</p> <p>3 we're -- so cancer as a complex disorder, you</p> <p>4 know, begins with an initiating event. But there</p> <p>5 is -- there is absolutely tumor evolution from</p> <p>6 that initial event through the progression of the</p> <p>7 disease.</p> <p>8 So to state that the -- in the initial</p> <p>9 inflammatory response to the tumor is -- is not</p> <p>10 causative to the continuation of the disease I</p> <p>11 think would be incorrect.</p> <p>12 MS. BROWN:</p> <p>13 Q The Penninkilampi authors -- to</p> <p>14 conclude our discussion here -- concluded that</p> <p>15 the paragraph you were looking at with the</p> <p>16 sentence "The potential mechanism by which</p> <p>17 genital talc is associated with an increased risk</p> <p>18 of ovarian cancer, hence, remains unclear," do</p> <p>19 you see that?</p> <p>20 A Yes.</p> <p>21 Q And this meta-analysis was published in</p> <p>22 January of 2018; correct?</p> <p>23 A Correct.</p> <p>24 Q And it is, in fact, cited in the</p>

67 (Pages 262 to 265)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 266</p> <p>1 majority of the plaintiff expert reports in this 2 litigation. Did you see that? 3 MS. O'DELL: 4 Object to the form. If you know that. 5 Don't speculate. 6 MS. BROWN: 7 Q That's why I asked "Did you see that?" 8 A So I didn't specifically look at if 9 this was referenced. I -- I certainly referenced 10 it. But I would also point out another important 11 part of the -- of this same reference, a -- about 12 halfway down the following paragraph, beginning 13 with "If chronic inflammation due to ascending 14 foreign bodies is indeed the mechanism by which 15 talc use is associated with ovarian cancer risks, 16 then these results fit the picture." 17 So I think the authors were both 18 describing some things that remain unclear but 19 also offering some comments that are supportive 20 of our earlier discussions today on this 21 mechanism. 22 Q And your opinion here today, Doctor, is 23 limited to the potential mechanism; right? 24 MS. O'DELL:</p>	<p style="text-align: right;">Page 268</p> <p>1 available data that there is a biologically 2 plausible mechanism surrounding and, indeed, in 3 the previous paragraph at the end of it where 4 they discuss use of -- or expression of 5 cyclooxygenase 1 and 2 as well as the action of 6 NSAIDs, again, supportive of -- somewhat 7 supportive of the inflammatory model. But... 8 MS. BROWN: 9 Q Well, as it relates to the NSAIDs, 10 Doctor, they point to the fact that the NSAID 11 data is inconsistent, at best, as evidence 12 supportive of their conclusions that the 13 mechanism is unclear; right? 14 A No. They point to it as -- they 15 actually try to clarify that the -- the seemingly 16 contradictory data regarding the NSAID use can be 17 explained by the relatively low expression of 18 cyclooxygenase 1 and cyclooxygenase 2, which are 19 the targets of most common NSAIDs. 20 Q What they say is that the use of 21 nonsteroidal anti-inflammatory drugs, NSAIDs, is 22 not inversely associated with the incidence of 23 ovarian cancer as may be expected if the etiology 24 was related to chronic inflammation. Right?</p>
<p style="text-align: right;">Page 267</p> <p>1 Object to the form. 2 A So my -- my opinion is -- is -- is 3 regarding a biologically plausible mechanism. 4 But, then -- and, in doing so, have reviewed some 5 of these studies that we're discussing now. 6 MS. BROWN: 7 Q Good. 8 And, as it relates to that potential 9 mechanism, these Penninkilampi authors conclude 10 that the potential mechanism remains unclear. 11 Right? 12 MS. O'DELL: 13 Objection to form. 14 A They -- the article makes a statement, 15 "The potential mechanism by which genital talc is 16 associated with an increased risk of ovarian 17 cancer, hence, remains unclear." 18 However, as we've been discussing, they 19 go on to state, "If chronic inflammation due to 20 ascending foreign body is indeed the mechanism," 21 then there -- the results in this paper 22 are -- fit that model. 23 So I think they're making reason- -- 24 making reasonable statements based on the</p>	<p style="text-align: right;">Page 269</p> <p>1 MS. O'DELL: 2 Objection to form. 3 A Yes, that statement is made. But, 4 importantly, it is incomplete without the next 5 sentence, again, explaining that -- that 6 apparent -- that apparent question. 7 So if the -- if NSAIDs are not 8 effective in ovarian cancer and the -- and, in 9 turn -- and if the observation is also made that 10 ovarian cancer cells don't express cyclooxygenase 11 1 and 2, then they would not -- they would be 12 nonresponsive to NSAIDs. 13 Q You state on page 12 of your report, 14 Doctor, in the last paragraph, the second-to-last 15 sentence that begins "moreover," that the effect 16 of nonsteroidal anti-inflammatory drugs, NSAIDs, 17 to reduce the risk of ovarian cancer provides 18 additional support for what you're discussing 19 here, which is that chronic inflammation plays a 20 key role in the development of ovarian cancer. 21 Right? 22 A Correct. 23 Q And that is, in fact, the opposite of 24 what the authors in Penninkilampi report as</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 270</p> <p>1 relates to NSAIDs; right?</p> <p>2 MS. O'DELL:</p> <p>3 Object to the form.</p> <p>4 A Not -- not necessarily. So there's --</p> <p>5 getting back to the -- the specific cells under</p> <p>6 question and the inflammatory response being</p> <p>7 examined. And, so, if we are lowering overall</p> <p>8 chronic inflammation through the use of an NSAID</p> <p>9 is -- is one question. A separate question is is</p> <p>10 a -- is a ovarian cancer cell responsive to</p> <p>11 NSAIDs. So they're two separate biological</p> <p>12 phenomenon.</p> <p>13 And, in one case, if those cells are</p> <p>14 not expressing the cyclooxygenase 1 and 2,</p> <p>15 they'll be nonresponsive.</p> <p>16 I would speculate that NSAID use in the</p> <p>17 rest of the body would still result in the</p> <p>18 expected effect due to, you know, the -- due to</p> <p>19 the inhibition of cyclooxygenase 1 and 2.</p> <p>20 So I don't think they're necessarily in</p> <p>21 conflict with each other.</p> <p>22 (DEPOSITION EXHIBIT NUMBER 20</p> <p>23 WAS MARKED FOR IDENTIFICATION.)</p> <p>24 MS. BROWN:</p>	<p style="text-align: right;">Page 272</p> <p>1 statement.</p> <p>2 And then there was, I think,</p> <p>3 importantly, the Lin 2011 paper is also relevant.</p> <p>4 Q Well, as it relates to the Merritt</p> <p>5 paper, this cite is wrong; right?</p> <p>6 A I need a moment to --</p> <p>7 Q Let's look at what Merritt actually</p> <p>8 found about pelvic inflammatory disease.</p> <p>9 If you look --</p> <p>10 MS. O'DELL:</p> <p>11 If you need a moment --</p> <p>12 Excuse me. I'm sorry. I didn't mean</p> <p>13 to interrupt you.</p> <p>14 If you need a moment to refresh</p> <p>15 yourself, Dr. Levy, please do.</p> <p>16 MS. BROWN:</p> <p>17 Q Sure. And if you -- when you're ready,</p> <p>18 Doctor, I'll direct you to the second column on</p> <p>19 page 174, and I want to talk about the last</p> <p>20 paragraph there that begins "if inflammation."</p> <p>21 A Page?</p> <p>22 Q And I'll read it into the record while</p> <p>23 you orient yourself. It's page 174, right-hand</p> <p>24 column. Final paragraph states, "If inflammation</p>
<p style="text-align: right;">Page 271</p> <p>1 Q Handing you what we've marked as</p> <p>2 Defense Exhibit 20 to your deposition, this is a</p> <p>3 paper by Merritt entitled "Talcum Powder Chronic</p> <p>4 Pelvic Inflammation and NSAIDs in Relation to the</p> <p>5 Risk of Epithelial Ovarian Cancer."</p> <p>6 Do you see that?</p> <p>7 A I do.</p> <p>8 Q And, in fact, on page 12 of your</p> <p>9 report, you cite this Merritt article. Correct?</p> <p>10 A Yes. Uh-huh.</p> <p>11 Q And you cite it for the proposition</p> <p>12 that studies have found a relationship between</p> <p>13 pelvic inflammatory disease and ovarian cancer</p> <p>14 risk. Correct?</p> <p>15 A Correct.</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 MS. BROWN:</p> <p>19 Q And you point to Merritt when you</p> <p>20 determine here as a finding of a relationship</p> <p>21 between pelvic inflammatory disease and ovarian</p> <p>22 cancer in support of your opinion that</p> <p>23 inflammation can cause ovarian cancer. True?</p> <p>24 A I'd have to double-check that</p>	<p style="text-align: right;">Page 273</p> <p>1 plays a role in the etiology of ovarian cancer,</p> <p>2 then it would be expected that PID would be</p> <p>3 associated with increased risks of ovarian</p> <p>4 cancer. PID is not associated with elevated risk</p> <p>5 of ovarian tumors in our data, confirming several</p> <p>6 previous reports of no association with PID in</p> <p>7 studies of all subtypes of ovarian cancer."</p> <p>8 Did I read that correctly?</p> <p>9 A You did.</p> <p>10 Q All right. So you cited this study for</p> <p>11 the proposition that studies have found a</p> <p>12 relationship between PID and ovarian cancer risk.</p> <p>13 Right?</p> <p>14 A No. I said -- I cited -- I said</p> <p>15 studies have found a relationship, yes, between</p> <p>16 PID and ovarian cancer risk.</p> <p>17 Q And, in fact, this study did not find a</p> <p>18 relationship between PID and ovarian cancer risk.</p> <p>19 Right?</p> <p>20 A I think this study found a -- I'm just</p> <p>21 looking at the...</p> <p>22 So -- I'm sorry. Would you ask your</p> <p>23 question again? This -- this study did not</p> <p>24 find your --</p>

69 (Pages 270 to 273)

Shawn Levy, Ph.D.

Page 274	Page 276
<p>1 Yes, I --</p> <p>2 Q Sure. I just -- you cited this study</p> <p>3 for the proposition that it showed there was a</p> <p>4 relationship between pelvic inflammatory disease</p> <p>5 and ovarian cancer risk, but, in fact, the study</p> <p>6 showed the opposite. Correct?</p> <p>7 A Well, to be clear on the wording,</p> <p>8 stated that the studies have found a</p> <p>9 relationship. I didn't indicate whether it was</p> <p>10 positive or negative.</p> <p>11 But I think, importantly, the study</p> <p>12 also has an important paragraph that is probably</p> <p>13 more related to its inclusion, which is on the</p> <p>14 same page we were just on, 174, second full</p> <p>15 paragraph in the discussion.</p> <p>16 Q One of the things on this page,</p> <p>17 Doctor --</p> <p>18 MS. O'DELL:</p> <p>19 Are you finished, Doctor?</p> <p>20 A I think important to at least finish</p> <p>21 that thought.</p> <p>22 That paragraph reads, "Focusing on talc</p> <p>23 use, we found that any use of perineal talc was</p> <p>24 associated with a small but significantly</p>	<p>1 quote, "We conclude that, on balance, chronic</p> <p>2 inflammation does not play a major role in the</p> <p>3 development of ovarian cancer."</p> <p>4 Q Do you see that, Doctor?</p> <p>5 A I see that.</p> <p>6 Q And what this study did was it</p> <p>7 endeavored to look into factors potentially</p> <p>8 associated with ovarian inflammation to see if it</p> <p>9 could support the theory that chronic</p> <p>10 inflammation plays a role in ovarian cancer;</p> <p>11 right?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form.</p> <p>14 A I would need to -- this one limitation</p> <p>15 of this particular paper is that it is connecting</p> <p>16 inflammation as evidenced by pelvic inflammatory</p> <p>17 disease and assuming that that source and type of</p> <p>18 inflammation would be -- the fact that there's</p> <p>19 not a direct association between -- or an</p> <p>20 increased risk of ovarian cancer in the presence</p> <p>21 of pelvic inflammatory disease; therefore,</p> <p>22 inflammation must not play a role in ovarian</p> <p>23 cancer. So that is their conclusions.</p> <p>24 MS. BROWN:</p>
Page 275	Page 277
<p>1 increased risk of ovarian cancer overall and</p> <p>2 specifically amongst the invasive and LNP serous</p> <p>3 tumors, although no clear dose response with</p> <p>4 increase in duration of use was identified. This</p> <p>5 finding is consistent with results of previous</p> <p>6 studies."</p> <p>7 So in the case of the report and the</p> <p>8 biologically plausible mechanism that's been</p> <p>9 supported by these studies, these studies</p> <p>10 differentiating the process of pelvic</p> <p>11 inflammatory disease doesn't ex- -- doesn't</p> <p>12 exclude or refute the inflammatory role or the</p> <p>13 role inflammation may play in ovarian cancer.</p> <p>14 Q What this study concludes is that, on</p> <p>15 balance, chronic inflammation does not play a</p> <p>16 major role in the development of ovarian cancer.</p> <p>17 Do you recall reviewing this in connection with</p> <p>18 your opinions in this case?</p> <p>19 MS. O'DELL:</p> <p>20 Object to the form. Misstates the</p> <p>21 exhibit.</p> <p>22 MS. BROWN:</p> <p>23 Counsel, I'll direct you to the last</p> <p>24 paragraph of the abstract on page 1 which reads,</p>	<p>1 Q Well, they looked at a bunch of</p> <p>2 different inflammatory conditions, didn't they?</p> <p>3 That was the focus of the study. The authors</p> <p>4 endeavored to look at a number of different</p> <p>5 pro-inflammatory factors and see if they</p> <p>6 influenced ovarian cancer. Do you recall</p> <p>7 reviewing that?</p> <p>8 A I do. I think -- but, more</p> <p>9 importantly, when we look at the -- their</p> <p>10 specific statements that are surrounding the</p> <p>11 mechanism we're discussing today, which has to do</p> <p>12 with talc exposure and perineal talc use, I think</p> <p>13 their -- their statements in that sense, which</p> <p>14 have already been read, quite stand on their own.</p> <p>15 So what this may indicate is a variety</p> <p>16 of types of inflammation do -- as present in</p> <p>17 other diseases, those individually do not or may</p> <p>18 not have a specific role in the progression of</p> <p>19 ovarian cancer.</p> <p>20 But it does not -- again, it does not</p> <p>21 mean that ovarian inflammation at the site of</p> <p>22 talc exposure in the ovary can't have a role in</p> <p>23 the progression of disease where -- again, as we</p> <p>24 were discussing earlier, with inflammation, we're</p>

70 (Pages 274 to 277)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 278</p> <p>1 now connecting independent biological processes.</p> <p>2 And I think you're -- I want to be sure</p> <p>3 we're clear and not drawing the use of the word</p> <p>4 "chronic inflammation" as meaning any</p> <p>5 inflammation and, therefore, if it's not</p> <p>6 associated with ovarian cancer, that inflammation</p> <p>7 can't have a role.</p> <p>8 What we're speaking about in terms of</p> <p>9 this mechanism is inflammation caused by the</p> <p>10 perineal use of talcum powder in the ovary and</p> <p>11 the -- and the -- to explain that increased risk</p> <p>12 of ovarian cancer, what is a plausible mechanism.</p> <p>13 Q The authors write, on page 74 -- 174,</p> <p>14 Doctor, second column, paragraph that begins with</p> <p>15 "It has been hypothesized," "It has been</p> <p>16 hypothesized that talc is linked to ovarian</p> <p>17 cancer development through inflammation," comma,</p> <p>18 "however evidence linking an inflammatory</p> <p>19 response with talc contamination of the ovaries</p> <p>20 is lacking."</p> <p>21 Do you see that?</p> <p>22 A I do.</p> <p>23 Q And you disagree with that statement?</p> <p>24 A I would -- I would suggest that a</p>	<p style="text-align: right;">Page 280</p> <p>1 couple times, and that's a 1.17 relative risk</p> <p>2 that you're referring to. Is that right?</p> <p>3 A Where is that?</p> <p>4 Q I'm looking at -- in the abstract.</p> <p>5 A Yes.</p> <p>6 Q Right. And the confidence interval is</p> <p>7 1.01 to 1.36. Right?</p> <p>8 A Correct.</p> <p>9 MS. O'DELL:</p> <p>10 As to what finding?</p> <p>11 MS. BROWN:</p> <p>12 The one we're discussing.</p> <p>13 Q And, Doctor, you know that one -- a</p> <p>14 confidence interval that begins with one is not</p> <p>15 statistically significant?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 MS. BROWN:</p> <p>19 Q Did you know that?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 A Well, I would say the authors have</p> <p>23 stated in that abstract that it is statistically</p> <p>24 significant.</p>
<p style="text-align: right;">Page 279</p> <p>1 number of studies in the literature since the</p> <p>2 publication of this paper would -- would suggest</p> <p>3 that these conclusions may have been premature.</p> <p>4 Q Do you think that, at the time this</p> <p>5 paper was published in 2008, that Merritt was</p> <p>6 accurately representing the data as it related to</p> <p>7 whether chronic inflammation could play a role in</p> <p>8 the development of ovarian cancer?</p> <p>9 MS. O'DELL:</p> <p>10 Object to the form.</p> <p>11 A I would say that Merritt has an</p> <p>12 unresolved -- has a number of unresolved</p> <p>13 conclusions or partial conclusions in their</p> <p>14 paper, again, including the paragraph we've</p> <p>15 discussed where they comment on the talc use with</p> <p>16 an increased risk of ovarian cancer.</p> <p>17 MS. BROWN:</p> <p>18 Q Did you see the confidence interval on</p> <p>19 that finding, Doctor?</p> <p>20 A I'd have to -- in --</p> <p>21 Is this in this paper or in the number</p> <p>22 of the --</p> <p>23 Q You reference the finding of an</p> <p>24 association between talc use and ovarian cancer a</p>	<p style="text-align: right;">Page 281</p> <p>1 MS. BROWN:</p> <p>2 Q Sure, because it's 1.01. My question</p> <p>3 to you was do you know that a confidence interval</p> <p>4 that begins with one is not statistically</p> <p>5 significant?</p> <p>6 This finding, Doctor, is barely</p> <p>7 statistically significant, isn't it?</p> <p>8 MS. O'DELL:</p> <p>9 Object to the form.</p> <p>10 A Again -- again, it's a -- whether it's</p> <p>11 barely or whether it's tremendously statistically</p> <p>12 significant, it -- it's still a finding that I</p> <p>13 would say is in support of -- has been supported</p> <p>14 by other studies with similar relative risk</p> <p>15 numbers in the -- in the 1.2 range and above, as</p> <p>16 indicated.</p> <p>17 MS. BROWN:</p> <p>18 Q Finally, Doctor, at the very -- the</p> <p>19 very last sentence of this Merritt study we're</p> <p>20 discussing, on page 175, concludes, "However,</p> <p>21 experimental evidence that perineal talc use</p> <p>22 elicits an inflammatory response in the ovaries</p> <p>23 is lacking, and overall we conclude that chronic</p> <p>24 inflammation does not play a major role in the</p>

71 (Pages 278 to 281)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 282</p> <p>1 development of ovarian cancer."</p> <p>2 And my question for you is what</p> <p>3 methodology did you employ to consider the</p> <p>4 findings of the Merritt paper in coming to your</p> <p>5 opinions contained in your report?</p> <p>6 MS. O'DELL:</p> <p>7 Object to the form.</p> <p>8 A Again, as we've discussed earlier here</p> <p>9 today, the -- there's been no singular paper that</p> <p>10 had a specific role in -- in developing the</p> <p>11 biologically plausible mechanism contained in the</p> <p>12 report. And, so, this -- this paper, among many</p> <p>13 others, was -- was used.</p> <p>14 MS. BROWN:</p> <p>15 Q Right. But the findings of this paper</p> <p>16 is that talcum powder doesn't produce an</p> <p>17 inflammatory response that leads to cancer.</p> <p>18 Right?</p> <p>19 A The -- the findings of this paper was</p> <p>20 that there's not an association of pelvic</p> <p>21 inflammatory disease and risk of ovar- -- of</p> <p>22 epithelial ovarian cancer.</p> <p>23 Q They conclude that chronic inflammation</p> <p>24 doesn't play a role in the development of ovarian</p>	<p style="text-align: right;">Page 284</p> <p>1 Again, the observations in this paper</p> <p>2 are regarding chronic inflammation and its -- and</p> <p>3 its major role in the development of ovarian</p> <p>4 cancer; and, again, in this -- in the specific</p> <p>5 individuals that they've looked at, it's in</p> <p>6 regards to pelvic inflammatory disease.</p> <p>7 And, so, as far as weighting that</p> <p>8 paper, it would be similar to other papers and</p> <p>9 other observations in the sense that it was --</p> <p>10 that the mechanism that is supported by a wide</p> <p>11 variety of work considers a history of -- history</p> <p>12 of work in the talc, inflammation, and ovarian</p> <p>13 cancer fields both in basic research and</p> <p>14 epidemiology to come up -- to come to the</p> <p>15 conclusions and mechanisms that are proposed.</p> <p>16 I don't -- I can't give you a specific</p> <p>17 weighting algorithm that was used on any -- any</p> <p>18 given paper.</p> <p>19 MS. BROWN:</p> <p>20 Q Did you consider Merritt's finding that</p> <p>21 evidence linking an inflammatory response with</p> <p>22 talc of the ovaries is lacking?</p> <p>23 A I certainly considered their -- I</p> <p>24 considered their statements in the -- in the</p>
<p style="text-align: right;">Page 283</p> <p>1 cancer; right?</p> <p>2 A I think they've -- they've extended</p> <p>3 that observation regarding pelvic inflammatory</p> <p>4 disease to that conclusion.</p> <p>5 But I think the studies that have come</p> <p>6 after this and other -- certainly other areas of</p> <p>7 review would suggest that those specific -- the</p> <p>8 wording of those specific statements may not be</p> <p>9 the most appropriate representation of the -- of</p> <p>10 the observations made in the -- in the Merritt</p> <p>11 paper.</p> <p>12 Q So did you weight the Merritt paper</p> <p>13 less than some other papers that came after it?</p> <p>14 Or how did you --</p> <p>15 What I'm trying to understand is your</p> <p>16 methodology for considering this paper, which</p> <p>17 seems to squarely conclude talc doesn't cause</p> <p>18 inflammation.</p> <p>19 MS. O'DELL:</p> <p>20 Object to the form.</p> <p>21 A I'm not -- so I would -- I would</p> <p>22 disagree that -- this paper does not make those</p> <p>23 conclusions that talc does not cause</p> <p>24 inflammation. What they --</p>	<p style="text-align: right;">Page 285</p> <p>1 paper. And I would question the dichotomy of</p> <p>2 the -- of some of their statements regarding talc</p> <p>3 risk to cancer.</p> <p>4 And the first question that would come</p> <p>5 to mind for this particular study is how they</p> <p>6 assessed talc-related inflammation in --</p> <p>7 specifically in the ovary. I don't recall seeing</p> <p>8 how they made that assessment.</p> <p>9 It, instead, seemed to me that their</p> <p>10 assessments were based on chronic inflammation as</p> <p>11 it related to other biological conditions and</p> <p>12 then extrapolating that to rate of ovarian</p> <p>13 cancer.</p> <p>14 Q How do you think one should measure</p> <p>15 talc-related inflammation in the ovary?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A Again, I wasn't asked to -- to provide</p> <p>19 that opinion. But I would reference the more</p> <p>20 recent Saed paper which -- and other molecular --</p> <p>21 and other molecular studies and certainly defer</p> <p>22 to Dr. Saed as an expert witness to discuss</p> <p>23 appropriate measurements for talc-related</p> <p>24 inflammation in the -- in the ovary or ovarian</p>

72 (Pages 282 to 285)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 286</p> <p>1 cells.</p> <p>2 MS. BROWN:</p> <p>3 Q Have you spoken with Dr. Saed?</p> <p>4 A I have not.</p> <p>5 Q Have you requested any information from</p> <p>6 Dr. Saed?</p> <p>7 A No, I have not.</p> <p>8 Q Have you -- would you hold to the same</p> <p>9 opinion if you did not consider the work of</p> <p>10 Dr. Saed?</p> <p>11 MS. O'DELL:</p> <p>12 Objection to form. Vague.</p> <p>13 A I -- the work of Dr. Saed is -- is a</p> <p>14 consideration among the wide variety of other</p> <p>15 literature contained in here. And Dr. Saed's</p> <p>16 work for in vitro analysis and the quantitation</p> <p>17 of specific reactive oxygen species is -- is a</p> <p>18 factor in and it is in support of the mechanism</p> <p>19 that I've proposed, which is that that mechanism</p> <p>20 does not rely on that study or any singular study</p> <p>21 for it to be valid.</p> <p>22 MS. BROWN:</p> <p>23 Q The mechanism you proposed, Doctor, is</p> <p>24 not yet generally accepted in the scientific</p>	<p style="text-align: right;">Page 288</p> <p>1 biologically plausible mechanism that was also</p> <p>2 peer-reviewed, and I would rely on or point you</p> <p>3 to a number of other expert reports, particularly</p> <p>4 in the epidemiology space from this case, where</p> <p>5 you'll find a great many parallels to -- to this</p> <p>6 case.</p> <p>7 So I, instead, would state</p> <p>8 independently myself and other respected</p> <p>9 scientists have essentially developed the same</p> <p>10 opinions regarding mechanism in this -- in this</p> <p>11 particular space.</p> <p>12 MS. BROWN:</p> <p>13 Q Is there another plaintiffs' expert</p> <p>14 that you're aware of who holds the same opinion</p> <p>15 as you do on biological plausibility?</p> <p>16 A Yes.</p> <p>17 Q Who's that?</p> <p>18 A Patricia Moorman, who is an</p> <p>19 epidemiologist whose report I had the opportunity</p> <p>20 to read yesterday.</p> <p>21 Q Is there -- and -- and even though</p> <p>22 she's an epidemiologist, Dr. Moorman has a view</p> <p>23 on biological plausibility? Is that right?</p> <p>24 MS. O'DELL:</p>
<p style="text-align: right;">Page 287</p> <p>1 community. Would you agree?</p> <p>2 MS. O'DELL:</p> <p>3 Object to the form.</p> <p>4 A I wouldn't have a basis for that</p> <p>5 opinion. As -- as we talked about earlier, I</p> <p>6 haven't shared this mechanism to ask for that</p> <p>7 opinion.</p> <p>8 MS. BROWN:</p> <p>9 Q You haven't published the proposed</p> <p>10 mechanism that is the subject of your report. Is</p> <p>11 that right?</p> <p>12 A That's right.</p> <p>13 Q You haven't discussed the proposed</p> <p>14 mechanism that is the subject of your report with</p> <p>15 any of your colleagues at HudsonAlpha; correct?</p> <p>16 A That's correct.</p> <p>17 Q So whether or not the proposed</p> <p>18 mechanism that is the subject of your report</p> <p>19 would be accepted by your peers in the scientific</p> <p>20 community, that's not something you have yet</p> <p>21 evaluated; correct?</p> <p>22 MS. O'DELL:</p> <p>23 Object to the form.</p> <p>24 A My -- I wasn't requested to provide a</p>	<p style="text-align: right;">Page 289</p> <p>1 Object to the form.</p> <p>2 A She has a view on --</p> <p>3 In her report was a -- a view on</p> <p>4 mechanism -- on mechanism, which included the</p> <p>5 discussion of inflammatory response and its role</p> <p>6 in ovarian cancer, which parallels this report.</p> <p>7 MS. BROWN:</p> <p>8 Q Do you consider your proposed mechanism</p> <p>9 that is the subject of your report to be a novel</p> <p>10 concept in the scientific world?</p> <p>11 MS. O'DELL:</p> <p>12 Object to the form.</p> <p>13 A Which part?</p> <p>14 MS. BROWN:</p> <p>15 Q Any part.</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A Again, I -- my -- the -- what was</p> <p>19 requested of me was not to develop a novel</p> <p>20 concept or even to describe an untested</p> <p>21 hypothesis. What was requested of me was to</p> <p>22 review the available literature and provide a</p> <p>23 biologically plausible mechanism for talc</p> <p>24 exposure to ovarian cancer. And, so, that's</p>

73 (Pages 286 to 289)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 290</p> <p>1 what -- that's what my report provides. 2 MS. BROWN: 3 Q Do you think there could be other 4 biologically plausible mechanisms by which talcum 5 powder would be associated with ovarian cancer? 6 A I haven't been asked to -- to make a 7 review related to other biological mechanisms. I 8 was asked to develop a biologically plausible 9 mechanism. And upon review of the totality of 10 the literature, this mechanism that -- that I've 11 presented and provided in the report is, in my 12 opinion, the correct mechanism. 13 Q Did you have complete autonomy in your 14 task to develop a biologically plausible 15 mechanism? 16 A Yes. 17 Q Were there any limitations on how you 18 should go about developing this biologically 19 plausible limita- -- mechanism? 20 MS. O'DELL: 21 Object to the form of the question to 22 the degree that the question seeks -- 23 MS. BROWN: 24 Form.</p>	<p style="text-align: right;">Page 292</p> <p>1 for you here, Doctor, is, were -- was there any 2 limitation placed on you that you relied on in 3 trying to develop your biologically plausible 4 mechanism? 5 MS. O'DELL: 6 What's allowed -- you're well aware of 7 this, counsel, I know -- that what's discoverable 8 is are there materials considered -- you can ask 9 him that -- was there assumptions that he was 10 asked to make -- that's discoverable -- and the 11 compensation. Those are the three things. Not 12 conversations between counsel and Dr. Levy. 13 So -- 14 MS. BROWN: 15 Counsel, you can instruct or we'll get 16 the judge. We do not have time for your 17 speeches. We're trying to finish up and let 18 other people -- other people ask questions. 19 MS. O'DELL: 20 That's straight from the rules. You're 21 well aware of that. 22 MS. BROWN: 23 So here's the question. If you want to 24 instruct, we'll take a break and get the judge.</p>
<p style="text-align: right;">Page 291</p> <p>1 MS. O'DELL: 2 No, no. If it goes to conversations 3 with counsel, it is not form. It is 4 attorney-client privilege and it's protected. 5 Work product privilege is protected. 6 And, so, Dr. Levy -- 7 MS. BROWN: 8 No. Counsel -- 9 MS. O'DELL: 10 Excuse me. Excuse me. I'm directing 11 my witness based on privilege, and I can do that. 12 To the degree that counsel is trying to 13 seek the substance of discussions you had with 14 counsel, those are protected, and I direct you 15 not to answer. 16 To the degree there's something in your 17 mind to respond that's not that, you may -- you 18 may respond. 19 MS. BROWN: 20 Q And as -- as counsel well knows, 21 because we've had this discussion earlier this 22 week, the federal rules allow discovery of any 23 material you relied on in forming your opinions. 24 And, so, my answer here -- my question</p>	<p style="text-align: right;">Page 293</p> <p>1 Q Did you rely on any instruction from 2 counsel regarding any limitations on how you were 3 to attempt to develop your biologically plausible 4 mechanism? 5 A No. I was -- I was not provided -- 6 there were no -- 7 I'm trying to make sure I answer to be 8 correct. But my very simple and direct answer is 9 the requests for the report were very succinct 10 and were given without limitation. 11 Q Did you try to develop any mechanism 12 that you rejected in connection with your report? 13 MS. O'DELL: 14 Object to the form. Vague. 15 A So I would best answer that by saying I 16 did not develop an initial mechanism and, 17 instead, began a literature review looking at the 18 available literature in talcum powder 19 inflammation in cancer, ovarian cancer, and then 20 in related subjects, and then, through the course 21 of that review, was able to synthesize the 22 opinion that you have, that we've been 23 discussing, in the report. 24 MS. BROWN:</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 294</p> <p>1 Q Do you consider the biologically</p> <p>2 plausible mechanism that is the subject of your</p> <p>3 report to be a hypothesis?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form. Asked and</p> <p>6 answered.</p> <p>7 A No, no. In fact, it is not. And</p> <p>8 it's -- I think it's very fundamentally different</p> <p>9 than a hypothesis.</p> <p>10 Because, again, to state, the</p> <p>11 activities that were undertaken was a review of</p> <p>12 the literature and then, based on that review, a</p> <p>13 mechanism that was biologically plausible. It is</p> <p>14 not hypothetical.</p> <p>15 MS. BROWN:</p> <p>16 Q Have you tested your biologically</p> <p>17 plausible mechanism?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A Tested in the sense of --</p> <p>21 So I would -- I would answer that as --</p> <p>22 in -- in my opinion, I would suggest that this</p> <p>23 has been tested based on following the completion</p> <p>24 of the report and reading other similarly derived</p>	<p style="text-align: right;">Page 296</p> <p>1 mechanism, you mean other experts in this</p> <p>2 litigation?</p> <p>3 MS. O'DELL:</p> <p>4 Object to the form. Misstates his</p> <p>5 testimony.</p> <p>6 A Other -- other material -- the</p> <p>7 materials that I was -- that I was provided.</p> <p>8 MS. BROWN:</p> <p>9 Q And those materials are in the form of</p> <p>10 other expert reports like yours; right?</p> <p>11 MS. O'DELL:</p> <p>12 Object to the form.</p> <p>13 A They are.</p> <p>14 MS. BROWN:</p> <p>15 Q Are you aware of any nonlitigation</p> <p>16 expert that has arrived at the same biologically</p> <p>17 plausible proposed mechanism as you?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A Well, I think -- yeah, in the sense --</p> <p>21 in the sense of the number of publications we've</p> <p>22 been discussing and some of the more recent both</p> <p>23 reviews and -- and Saed's paper, I suppose, as</p> <p>24 we've been discussing, Dr. Saed has been funded</p>
<p style="text-align: right;">Page 295</p> <p>1 or similarly requested both literature, some of</p> <p>2 the publications that we've been discussing, as</p> <p>3 well as other expert reports that have, as we've</p> <p>4 just discussed, some parallel aspects.</p> <p>5 So, from a formal scientific process,</p> <p>6 that is -- would not, I think, be considered a</p> <p>7 formal test. But from the perspective of this</p> <p>8 biologically plausible mechanism, other</p> <p>9 scientists undertaking similar methodology came</p> <p>10 up with similar results.</p> <p>11 And, so, therefore, I would say that</p> <p>12 this report is -- continues to be supported by</p> <p>13 independent reviews and content.</p> <p>14 MS. BROWN:</p> <p>15 Q The other scientists that you just</p> <p>16 referenced are also paid experts for the</p> <p>17 plaintiffs; is that right?</p> <p>18 MS. O'DELL:</p> <p>19 Object to the form.</p> <p>20 A I don't have knowledge of that</p> <p>21 specifically.</p> <p>22 MS. BROWN:</p> <p>23 Q Well, when you said other experts</p> <p>24 looking at the same thing came up with a similar</p>	<p style="text-align: right;">Page 297</p> <p>1 for some of this work, but I would counter that</p> <p>2 with sponsorship of -- of studies that are</p> <p>3 subsequently peer-reviewed, I think are generally</p> <p>4 held to a scientific standard and rigor, and</p> <p>5 would suggest that his most recent work would</p> <p>6 fall under that and -- and, therefore, I would</p> <p>7 not consider that in the same realm as an expert</p> <p>8 report.</p> <p>9 MS. BROWN:</p> <p>10 Q Are you aware that the plaintiffs'</p> <p>11 lawyers funded Dr. Saed's studies?</p> <p>12 A I am.</p> <p>13 Q How do you know that?</p> <p>14 MS. O'DELL:</p> <p>15 Don't speculate. If you know it,</p> <p>16 testify to it.</p> <p>17 A No. I'm thinking of --</p> <p>18 That was disclosed during the</p> <p>19 discussion of the -- of the paper, and the</p> <p>20 question I asked and actually looked on the paper</p> <p>21 was to --</p> <p>22 And this -- this was getting to my own</p> <p>23 opinion as to the appropriateness and the</p> <p>24 potential scientific rigor of the paper, and that</p>

75 (Pages 294 to 297)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 298</p> <p>1 was whether or not Dr. Saed disclosed that 2 relationship, which is, of course, ethically a 3 requirement for sponsored research. And, indeed, 4 that sponsorship is made in the paper. 5 MS. BROWN: 6 Q Was it important to you -- 7 Did you ask Dr. Saed about the funding 8 for his paper? 9 A I did not. As we -- as we discussed, I 10 haven't spoken with him. 11 Q Were you troubled by the fact that 12 Dr. Saed's disclosure does not reference which 13 side of the litigation he's working for? 14 MS. O'DELL: 15 Object to the form. 16 A Are you asking for my opinion on if it 17 troubled me? 18 MS. BROWN: 19 Q Yeah. 20 A No. 21 Q It sounds like you did a little 22 investigation and you were satisfied with the 23 disclosure. Was that your testimony? 24 MS. O'DELL:</p>	<p style="text-align: right;">Page 300</p> <p>1 Q Why is it important, in your mind, to 2 disclose funding for a study? 3 A Well, it's, you know, ethical premise 4 of -- of most scientific research or really all 5 extramurally funded research that the funding 6 sources are -- are always disclosed. And that's 7 true for publication as well as presentation. 8 And, so, I think most -- most 9 scientists, during presentation, will present a 10 slide that shows their -- their funding support 11 and all of its sources regard- -- whether it's 12 public or private. 13 And then you'll notice in vast majority 14 of publications, if they are grant supported, 15 again, whether that grant is from a public or a 16 private institution, those things are referenced. 17 And, in fact, the U. S. Government has a 18 requirement that grants be referenced in their -- 19 in any publications that were supported by that 20 money. 21 Q Do you have any critiques of either of 22 Saed's papers? 23 A No. Not at this time. 24 Q Do you have any questions or anything</p>
<p style="text-align: right;">Page 299</p> <p>1 Object to the form. He didn't use the 2 word "investigation." 3 A I was satisfied seeing a disclosure 4 made regarding funding, which, again, in the 5 scientific climate I would -- or I would state 6 simply I viewed the support of that study which 7 subsequently goes out to peer review functionally 8 equivalent to pharmaceutical support of a study 9 involving a drug or a condition or a treatment. 10 The reality of the scientific space 11 is -- is -- is funding sponsorship comes from a 12 variety of cases. And in each institution, 13 HudsonAlpha certainly, I'm positive Wayne State 14 has a conflict of interest review board which 15 Dr. Saed has to report to as far as the -- how he 16 manages that potential conflict of interest. And 17 given that he's at a reputable institution that 18 I've actually done a fair amount of review work 19 with over the years, being Wayne State, I'm 20 reasonably -- or I would say I'm quite confident 21 that his conflict of interest has been managed 22 appropriately for the -- for the study that was 23 reviewed. 24 MS. BROWN:</p>	<p style="text-align: right;">Page 301</p> <p>1 that doesn't make sense to you, having reviewed 2 the most recent one or the 2017 one? 3 A No. My focus, particularly on the most 4 recent one, I actually found his molecular 5 studies to be quite comprehensive and -- 6 So there was -- there was no specific 7 concerns that -- that I was able to identify. 8 And, again, the -- in the -- in the version of 9 the paper that -- that I -- that I was given. 10 Q And did you have any opportunity to 11 check to see if you had an earlier version of 12 that paper? 13 A Oh, I -- I'll be sure and do that at 14 the next break. 15 Q Okay. Why don't we go ahead and take a 16 break now. You'll take a look, if you wouldn't 17 mind, to see if you have something other than 18 what we've marked at the deposition. 19 I'm going to renew -- review my notes. 20 I'm close to finishing, and then I'll hand it 21 over to my colleague, Mr. Ferguson, who I think 22 will have some questions for you as well. Okay, 23 Doctor? 24 A Uh-huh.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 302</p> <p>1 Q Thank you, Doctor. 2 VIDEOGRAPHER: 3 Going off the record. The time is 3:33 4 p.m. 5 (OFF THE RECORD.) 6 VIDEOGRAPHER: 7 We're back on the record. The time is 8 3:48 p.m. 9 MS. BROWN: 10 Q Welcome back, Doctor. 11 Did you have an opportunity to take a 12 look if you had an earlier version of Dr. Saed's 13 manuscript? 14 A I did. 15 I did not. 16 Q Okay. And, so, during this deposition, 17 you've referred from time to time to Dr. Saed's 18 2018 paper. Is that right? 19 A (Nods affirmatively.) 20 MS. O'DELL: 21 Object to the form. Excuse me. 22 MS. BROWN: 23 Q And you received that paper after you 24 authored your report in this case; right?</p>	<p style="text-align: right;">Page 304</p> <p>1 MS. BROWN: 2 Q And if that's not the one you were 3 thinking of, Doctor, we can move on. 4 A I was thinking Henderson 1971. 5 Q And that's not an animal study; right? 6 A Maybe this -- this isn't the same one, 7 then. I can certainly find it at the end if -- 8 The -- it was a 1971 study involving a 9 rat model that the major point and conclusion of 10 the study was perhaps something that we've 11 discussed that's been now well accepted that the 12 talc can migrate, after exposure, into the 13 ovarian tissue. 14 Q Are you aware of any study, Doctor, 15 that talc on the exterior of a woman's vagina can 16 migrate up the fallopian tubes to the ovary? 17 MS. O'DELL: 18 Object to the form. 19 A I am not aware of a study that tested 20 that specifically. 21 MS. BROWN: 22 Q And did you consider, in connection 23 with your opinions here, IARC's finding that the 24 science regarding migration is, quote, "weak"?</p>
<p style="text-align: right;">Page 303</p> <p>1 MS. O'DELL: 2 Object to the form. 3 A So I was referring -- 4 Yes. I -- I -- the manuscript we were 5 discussing was received after the completion of 6 this. But, as we discussed earlier, the 7 materials in the paper were presented in abstract 8 form or long abstract form, and those are 9 referenced in the report. 10 MS. BROWN: 11 Q And just to close the loop on one thing 12 before I hand it over to my colleague, 13 Mr. Ferguson, you had referenced an animal study 14 by Woodruff earlier in the day. Do you remember 15 that? 16 A Yes. 17 Q That paper doesn't have anything to do 18 with talc; right? 19 MS. O'DELL: 20 Object to the form. 21 A Let me -- 22 Yes, I -- you're -- the Woodruff 1979 23 paper is not the one I was -- I was wrong on the 24 author. Give me a moment to...</p>	<p style="text-align: right;">Page 305</p> <p>1 MS. O'DELL: 2 Object to the form. 3 A My -- my primary consideration of IARC 4 was their classification of the talc and the -- 5 and the fibrous talc, and I don't recall their 6 conclusions of the migration science being weak. 7 And, in fact, it appears, as stated by 8 the FDA, that the -- the migration question is -- 9 is well resolved. 10 MS. BROWN: 11 Q Finally, Doctor, in connection with 12 your opinions in this case, did you consider 13 articles regarding whether stick lesions evidence 14 inflammation? 15 A I'd have to review some of the 16 literature for stick lesions specifically. But 17 that -- 18 Can you -- what are you referring to by 19 stick lesions? 20 Q So do you understand that it's now 21 believed, in terms of the -- where ovarian cancer 22 begins, that it begins in the fallopian tubes, 23 epithelial ovarian cancer? 24 A I certainly would agree that a -- the</p>

77 (Pages 302 to 305)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 306</p> <p>1 site of initiation, whether -- that it can begin 2 in the fallopian tubes, yes, that there's been 3 studies that have shown that evidence. 4 Q And some of the early lesions that have 5 been found in the fallopian tubes are sometimes 6 referred to as stick lesions. Are you familiar 7 with that? 8 MS. O'DELL: 9 Object to the form. 10 A I'm not. 11 MS. BROWN: 12 So you haven't looked at any studies 13 that have looked at stick lesions that have been 14 removed from women to see if there was any 15 evidence of inflammation? 16 MS. O'DELL: 17 Object to the form. 18 A That -- that -- I don't recall that as 19 part of the review. 20 MS. BROWN: 21 Q Fair enough. 22 No further questions. I'll hand it 23 over to Mr. Ferguson. 24 MR. FERGUSON:</p>	<p style="text-align: right;">Page 308</p> <p>1 the Genomic Services Laboratory -- 2 Right? There's one of those at 3 HudsonAlpha; right? 4 A There is. 5 Q Do you perform services there such as 6 running clinical samples to report results to 7 healthcare providers? Is that the kind of things 8 you do? 9 A To be -- to be clear and to, 10 importantly, differentiate the regulated lab 11 versus the research laboratory, the Genomic 12 Services Laboratory is a -- is a entity of 13 HudsonAlpha that is responsible for research 14 activities. 15 There is a separate wholly owned 16 subsidiary of HudsonAlpha creatively named the 17 Clinical Services Laboratory. So that laboratory 18 is the laboratory that performs the testing. And 19 to hopefully not provide a level of confusion, 20 but the two laboratories coexist in the same 21 space. And what this means is I have staff and 22 equipment. Some is dedicated to clinical, some 23 is dedicated to research, and some are shared 24 between the two.</p>
<p style="text-align: right;">Page 307</p> <p>1 Thank you. 2 EXAMINATION 3 BY MR. FERGUSON: 4 Q Good afternoon, Dr. Levy. My -- my 5 name is Ken Ferguson, and I represent Imerys in 6 this matter. Do you know who Imerys is? 7 A Only that they're a mining company. 8 Q Okay. And I have some questions for 9 you. I apologize for my voice. I've kind of had 10 my allergies and then going into a cold, so it's 11 kind of -- kind of stuffy. So I apologize. 12 If you have trouble hearing me or 13 understanding me, let me know. Okay? 14 A Okay. 15 Q And -- and just -- I know you've been 16 at this with Miss Brown for a little while, but 17 if there's any question that you don't understand 18 that I'm asking you, just let me know, and I'll 19 restate it so I can make sure that we're 20 communicating. Okay? 21 A Okay. 22 Q I want to talk to you, first of all, 23 about a little bit more about what you do at 24 HudsonAlpha Institute. So in the what's called</p>	<p style="text-align: right;">Page 309</p> <p>1 So, in summary, the best way to 2 consider the laboratory is that it's a clinical 3 regulated laboratory that also performs research. 4 Any projects under that research 5 umbrella are referred to as being in the Genomic 6 Services Laboratory. Anything clinical is 7 referred to the Clinical Services Laboratory. 8 That lab has been CLIA-licensed now for going on 9 five -- just past four years and has been 10 CAP-accredited for three and a half. 11 Q So is it the Clinical Services 12 Laboratory, then, that would perform services 13 like running clinical samples to get results to 14 healthcare providers? 15 A That's correct. 16 Q And -- and among those things that the 17 Clinical Services Laboratory does, is that 18 restricted to whole genome sequencing? 19 A Our currently -- the only publicly 20 disclosed and validated test for the Clinical 21 Services Laboratory is whole genome sequencing. 22 We have two other laboratory-developed 23 tests, or commonly referred to as LDTs, that are 24 run in a -- as a private assay for some clinical</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 310</p> <p>1 trials, so they're not publicly available and to 2 date have not been publicly disclosed. They're 3 protected under confidentiality agreement. 4 And the Clinical Services Laboratory 5 this year will launch a number of other tests 6 that we have publicly disclosed. Those include 7 whole exome sequencing, an oncology panel known 8 as the TruSight Tumor 170, which profiles 170 9 genes with -- that have been -- that have known 10 involvement in cancer risk and progression, and 11 as well as a 500 panel of similar form. 12 Q So let me talk to you a little bit 13 about your prior position. You were at 14 Vanderbilt University Medical Center; correct? 15 A Correct. 16 Q And you were an assistant professor? 17 Is that correct? 18 A The titles I held there was research 19 assistant professor and then assistant professor, 20 and then I was a associate professor as an 21 adjunct faculty for a number of years after 22 joining HudsonAlpha. So I had to progress 23 through a few of the academic ranks at 24 Vanderbilt, but all of them in the professor</p>	<p style="text-align: right;">Page 312</p> <p>1 of pre-reviews for tenure. There were no 2 concerns with that progress. But, based on both 3 funding as well as publication records, I wasn't 4 overly concerned with that. 5 But the opportunity to be able to do -- 6 and the scale of operations at HudsonAlpha was -- 7 was too good to turn down, as far as remaining at 8 Vanderbilt. 9 Q So you were neither granted tenure nor 10 denied tenure. Is that fair to say? 11 A That's fair to say. 12 I think the best evidence for the 13 relationship at Vanderbilt after my leaving was I 14 continued as an adjunct faculty in the same 15 department, again with change in title, for a 16 number of years after joining HudsonAlpha. So it 17 was a -- certainly, I wouldn't characterize it as 18 a negative departure from the institution. And I 19 still remain a collaborator with a number of 20 colleagues there. 21 Q Do you have a copy of your report in 22 front of you? 23 A I do. 24 Q Okay. What I'm gonna do is I'm gonna</p>
<p style="text-align: right;">Page 311</p> <p>1 realm. 2 Q As an assistant professor, were you 3 appointed on a tenure track? 4 A Yes. 5 Q And do you know generally how many 6 years after appointment as an assistant professor 7 is a tenure decision at Vanderbilt typically made 8 in that department? 9 A It varies from probably five to nine. 10 Q Did you ever achieve tenure at 11 Vanderbilt? 12 A Actually, I was up for tenure the year 13 that I moved to HudsonAlpha. 14 Q So -- 15 A So, technically, I, which will sound 16 odd, I was promoted to associate professor upon 17 leaving. 18 Q Okay. 19 A In an adjunct role. 20 Q So were you turned down for tenure 21 or -- 22 A I was not. I never -- I -- the 23 opportunity at HudsonAlpha predated the time that 24 I would have gone up for tenure. I had a number</p>	<p style="text-align: right;">Page 313</p> <p>1 try to go through, probably in -- in order, 2 portions of your report that I want to ask about 3 and try to make sure I don't cover things that 4 Miss Brown's already covered. 5 Can you look at page 5 of your report? 6 A Yes. 7 Q So there -- and I'm looking at number 2 8 on page 5, Acquired Somatic Gene Mutation. 9 Do you see that? 10 A I do. 11 Q And you say there that -- 12 I'm skipping the sentences. If you 13 need to go back, feel free. 14 -- "Biological and lifestyle exposures, 15 such as viruses, obesity, hormones and chronic 16 inflammation, are also known to result in 17 cancer-causing mutations." 18 Right? 19 A I see that sentence. 20 Q Okay. Wouldn't you agree that the 21 association between obesity and cancer risk is 22 just that, an association and not a known 23 cause-and-effect relationship? 24 MS. O'DELL:</p>

Shawn Levy, Ph.D.

Page 314	Page 316
<p>1 Object to the form.</p> <p>2 A I would state that it is known that</p> <p>3 cancer rates increase in a number of unhealthy</p> <p>4 conditions, including obesity. But I am not</p> <p>5 aware of a -- of any studies that have</p> <p>6 illustrated a causal effect directly between</p> <p>7 obesity and cancer.</p> <p>8 MR. FERGUSON:</p> <p>9 Q And, specifically, isn't it true that</p> <p>10 there is no direct in vivo experimental evidence</p> <p>11 that obesity causes cancer-causing mutations?</p> <p>12 A I would have to review the literature</p> <p>13 to -- before answering that question. But the</p> <p>14 relationship between obesity and cancer risk</p> <p>15 is -- is quite well established. And I think for</p> <p>16 us to discuss that in more detail, we'd have to</p> <p>17 start delving into some of the specifics around</p> <p>18 the physiological changes related to obesity and</p> <p>19 whether those specific physiological changes play</p> <p>20 a role in cancer.</p> <p>21 Q And, just below that, the last sentence</p> <p>22 in that paragraph, you say, "These mechanisms may</p> <p>23 be direct, such as radiation directly damaging</p> <p>24 DNA, as well as indirect, such as an external</p>	<p>1 A It varies. So the -- the --</p> <p>2 "inflammatory response" is a bit general. So</p> <p>3 depending on specific type of cellular</p> <p>4 recruitment and cellular damage through the</p> <p>5 release of cytokines, the release of oxidative</p> <p>6 damaging materials from cells like granulocytes,</p> <p>7 you know, or the -- even the cell's own</p> <p>8 production of reaction to -- reactive oxygen</p> <p>9 species, such as from the mitochondria, which is</p> <p>10 the most common sync -- or most common source of</p> <p>11 reactive oxygen species in the cell.</p> <p>12 And, so, those are some examples of --</p> <p>13 of that relationship between an inflammatory</p> <p>14 response and that cellular reaction.</p> <p>15 Q Reactive oxygen species are not the</p> <p>16 same thing as inflammation; correct?</p> <p>17 A I would say reactive oxygen species are</p> <p>18 a hallmark of inflammation.</p> <p>19 Q But they're not the same thing.</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 A The -- well, they are --</p> <p>23 Again, reactive oxygen species are a</p> <p>24 component of inflammation. So they're -- the</p>
Page 315	Page 317
<p>1 agent causing a cellular -- cellular reaction or</p> <p>2 inflammatory response that then leads to DNA</p> <p>3 damage or mutation."</p> <p>4 What cellular reactions are you</p> <p>5 referring to that result in DNA damage or</p> <p>6 mutation?</p> <p>7 A So the presence of reactive -- so a few</p> <p>8 different things. Primarily, along the</p> <p>9 discussions for today, the presence of reactive</p> <p>10 oxygen species which can directly -- which are a</p> <p>11 cellular reaction that can then cause -- directly</p> <p>12 cause DNA damage.</p> <p>13 There's protein oxidation effects that</p> <p>14 are similar to that, in the sense that you have a</p> <p>15 chemical change and a cellular component that</p> <p>16 results in a -- in a protein activity change,</p> <p>17 again leading to potential DNA damage.</p> <p>18 And then you can have --</p> <p>19 So those are two -- two examples of</p> <p>20 cellular reactions to that.</p> <p>21 Q And -- and maybe you just explained it,</p> <p>22 but I wanted to make sure I'm clear. What is the</p> <p>23 mechanism by which an inflammatory response</p> <p>24 results in DNA damage?</p>	<p>1 words are two -- two different definitions, but</p> <p>2 they are a component.</p> <p>3 MR. FERGUSON:</p> <p>4 Q Would you agree that reactive oxygen</p> <p>5 species are a normal part of cell physiology?</p> <p>6 A Yes, absolutely.</p> <p>7 Q And the major source of reactive oxygen</p> <p>8 species comes from inside the cell and is</p> <p>9 produced in mitochondria?</p> <p>10 A A source, and depending on the site of</p> <p>11 the physiology. So a normal, healthy cell not</p> <p>12 under stress or injury would be -- then, yes,</p> <p>13 that's a true statement.</p> <p>14 Under different physiological</p> <p>15 conditions, that statement may not be true.</p> <p>16 Q Can you distinguish reactive oxygen</p> <p>17 species produced inside a cell from reactive</p> <p>18 oxygen species produced outside the cell?</p> <p>19 A What do you mean? So by -- by</p> <p>20 "distinguish," you mean --</p> <p>21 Q Can you tell the difference?</p> <p>22 A I'm just thinking if there's a way to</p> <p>23 measure.</p> <p>24 So you can measure the effects of</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 318</p> <p>1 exogenously introduced reactive oxygen species 2 and then compare that to the measurement of 3 endogenously produced reactive oxygen species. 4 But as far as determining the 5 difference if the cellular integrity is not 6 intact, I'm not aware of a method to do that. 7 Q Would you agree that generation of 8 reactive oxygen species is an inevitable 9 consequence of aging in aerobic organisms? 10 MS. O'DELL: 11 Object to the form. 12 A So reactive oxygen species are a -- 13 are present at all stages of life. And aging, as 14 a biological phenomenon, is probably one of the 15 most variable phenomenon that exists. 16 And specific to reactive oxygen 17 species, the diet, lifestyle, and genetics of 18 that individual will drastically change that. 19 And a new area of research that my 20 laboratory has been undertaking for a short 21 time -- 22 And, so, I don't have specific 23 publications, and it's really not -- I promise 24 it's not taking us too far afield.</p>	<p style="text-align: right;">Page 320</p> <p>1 would be very difficult. 2 MR. FERGUSON: 3 Q In your report, on this same page, you 4 discuss the fact that, even if someone has a 5 genetic mutation that predisposes them to cancer 6 doesn't mean that he or she is certain to get 7 cancer. Correct? 8 A That is correct. 9 Q So there is a -- a random component to 10 the effects of known cancer-causing agents. 11 Right? 12 MS. O'DELL: 13 Objection to form. 14 A There is a complicated relationship 15 between genetics, environment, and expose -- or 16 environment, including exposure and lifestyle, 17 and the progression of cancer. 18 Perhaps the -- a summary analogy is the 19 more predisposing mutations that an individual 20 has, it's -- it's equivalent to their body is 21 rolling the dice more often to collect a mutation 22 sufficient to cause cancer than somebody who does 23 not have the same genetic background. 24 And there's -- there's many, many lines</p>
<p style="text-align: right;">Page 319</p> <p>1 -- but is the concept of your annual 2 age versus biological age. And my lab has some 3 assays that are based on epigenetics as well as 4 some metabolomic markers. And what we found -- 5 now, in very, again, preliminary data -- that 6 individuals will vary by plus or minus 15 years 7 from physiological age to annual age based on, 8 again, a number of lifestyle factors not 9 important for this study. 10 But the point I'm making is the 11 discussion about level of reactive oxygen species 12 and its association with age is actually quite 13 variable based on the long -- or based on the 14 current physiological activity of that person. 15 Stated very simply, which is probably 16 something we all know, the better shape you're 17 in, the younger your physiology will appear. And 18 you can actually modulate that quite quickly, 19 meaning that a person who's 60 and has made poor 20 lifestyle choices can actually gain back quite a 21 bit of that physiological age quite quickly. 22 And so, again, to directly answer your 23 question, a annual age-related conclusion 24 regarding production of reactive oxygen species</p>	<p style="text-align: right;">Page 321</p> <p>1 of evidence. Probably the most prominent is 2 BRCA1 and 2 mutation and the role it plays in 3 increased risk of breast and ovarian cancer. 4 MR. FERGUSON: 5 Q Wouldn't you agree that even the 6 inherited susceptibility cannot entirely explain 7 this random component of some people getting 8 cancer when exposed and some people not? 9 MS. O'DELL: 10 Objection to form. 11 A DNA -- so that, it's very 12 gene-dependent. So BRCA1 and 2 is the example 13 given. That is correct, that if you have a BRCA1 14 and -- 1 or 2 mutation, you are not guaranteed to 15 get cancer. 16 Corollary to that is if you do not have 17 a BRCA1 and 2 mutation, your relative risk for 18 cancer does not change, meaning that you're at no 19 less of a risk than somebody -- somebody else who 20 doesn't have that mutation. 21 I should state that there are other 22 genes. P53 is a good example that was mentioned 23 earlier. If you carry a mutation in that gene, 24 the probability that you'll get cancer, assuming</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 322</p> <p>1 you don't die from something else, is almost 2 certain, meaning that it's in the mid to high 90 3 percents if you -- if you live until a late age. 4 MR. FERGUSON: 5 Q Further down this paragraph, you 6 indicate that "An inherited gene mutation could 7 instead make one more likely to develop cancer 8 when exposed to certain cancer-causing 9 substances." 10 Correct? That's your statement? 11 A Yes. 12 Q Can you provide any examples in which a 13 woman with an inherited mutation in a particular 14 gene has been demonstrated to have more 15 sensitivity to developing ovarian cancer as a 16 result of exposure to an environmental agent? 17 A Not for ovarian cancer specifically. I 18 would need to review -- 19 There is a -- I've seen report of a 20 single gene related to ovarian cancer, which, 21 again, I would have to do a bit of searching to 22 be sure I'm naming the correct gene, but I -- 23 where that has a much high- -- increased risk 24 specific to ovarian cancer, but I do not recall</p>	<p style="text-align: right;">Page 324</p> <p>1 And the point of my mentioning this is 2 to illustrate that an early predisposition to -- 3 or a significant predisposition to cancer that 4 results in a early cancer event, those 5 individuals show a lifetime increase in risk of 6 approximately -- they're -- they're approximately 7 six times, depending on the disease, to 13 times 8 more likely to get that -- to get a secondary 9 disease. 10 So there clearly is a relationship to 11 predisposition in -- in oncology -- or in rate of 12 cancer event. 13 Q Okay. And I appreciate your response. 14 But remember that my question was related to 15 ovarian cancer, and -- and we went a little 16 afield from ovarian cancer. 17 And I want to ask you another question 18 in that regard. Can you provide any example in 19 which a woman with an inherited mutation in a 20 particular gene has been demonstrated to have 21 more sensitivity to developing ovarian cancer as 22 a result of exposure to talcum powder? 23 MS. O'DELL: 24 Object to the form.</p>
<p style="text-align: right;">Page 323</p> <p>1 if there was a measurement of any exogenous 2 exposure risk that amplified that effect or not. 3 But I think the -- as a general 4 premise, it is a -- well established in cancer 5 biology that any mu- -- any mutation that results 6 in a burden related to DNA repair, related to 7 cell cycle control, you are more susceptible to 8 cancer. 9 In one of our lines of research where 10 we do have some publications, in pediatric 11 cancer, I would simply point to in approximately 12 50 percent of adults who are survivors of 13 childhood cancer will develop a second cancer 14 event primarily because their -- the fact that 15 they developed a childhood cancer generally means 16 you are predisposed to that condition. 17 And -- and, as evidenced in the 18 observations we've done in the analysis of 19 thousands of patients in collaboration with 20 St. Jude and the children's oncology group, we've 21 identified now a ability to do genetic counseling 22 in those individuals and predict with very high 23 accuracy what their secondary cancer is likely to 24 be.</p>	<p style="text-align: right;">Page 325</p> <p>1 Answer the question. 2 A So the mechanism we proposed would be 3 independent of -- of that predisposition. But I 4 would have the opinion that an individual with 5 any predisposition mutation, regardless of the 6 gene but -- and -- in ovarian cancer, that they 7 would be a more fragile individual as -- when it 8 comes to this exposure under the mechanism that 9 we've been discussing today. 10 MR. FERGUSON: 11 Q Okay. And what I'm looking for is some 12 example or some literature in that regard. 13 A I would -- I would have to -- I would 14 have to look -- 15 Q Okay. 16 A -- to see. 17 Q So what you've told me is that's your 18 opinion, but you don't have any references for it 19 as you sit here? 20 MS. O'DELL: 21 Objection to form. 22 A So my -- what was -- I was requested to 23 provide this biologically plausible mechanism, 24 and part of that request was not necessarily</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 326</p> <p>1 include the influence on that mechanism that</p> <p>2 specific gene mutations or inherited risks may</p> <p>3 have within relation to ovarian cancer.</p> <p>4 So I'd certainly be delighted to pause</p> <p>5 for a moment and take -- you know, and -- and</p> <p>6 work on that -- give you that -- see if I can</p> <p>7 give you that specific example.</p> <p>8 MR. FERGUSON:</p> <p>9 Q But you can't as you sit here?</p> <p>10 A I cannot.</p> <p>11 Q Okay. So let's look at -- further down</p> <p>12 on page 5, you have a section entitled "The Role</p> <p>13 of Genetics in Ovarian Cancer." Correct?</p> <p>14 A Correct.</p> <p>15 Q And I want to look at a reference that</p> <p>16 you -- you have cited. And let me mark this as</p> <p>17 an exhibit, please. I guess I can mark it.</p> <p>18 (DEPOSITION EXHIBIT NUMBER 21</p> <p>19 WAS MARKED FOR IDENTIFICATION.)</p> <p>20 MR. FERGUSON:</p> <p>21 Q Exhibit 21 is the Nunes article. Have</p> <p>22 you seen that?</p> <p>23 A I have, yes.</p> <p>24 Q Okay. So if we look at page 5, at top</p>	<p style="text-align: right;">Page 328</p> <p>1 further and you have a sentence that starts</p> <p>2 "epithelial ovarian cancer." Correct?</p> <p>3 MS. O'DELL:</p> <p>4 On page 6 there?</p> <p>5 MR. FERGUSON:</p> <p>6 Yeah. I apologize. Yeah, it is.</p> <p>7 A Yep.</p> <p>8 MR. FERGUSON:</p> <p>9 Q It's on page 6. It's the, I believe,</p> <p>10 the last sentence of the partial paragraph at the</p> <p>11 top of 6. See it?</p> <p>12 A I do.</p> <p>13 Q Okay. And you say, "Epithelial ovarian</p> <p>14 cancer (EOC) includes most malignant ovarian</p> <p>15 neoplasms" -- you cite Chan, 2006 -- "that can be</p> <p>16 classified based on morphologic and molecular</p> <p>17 genetic features into the following types:</p> <p>18 Serous" -- and, in parentheses, "(OSC) low and</p> <p>19 high grade); endometrioid (EC), clear cell,</p> <p>20 (OCCC), and mucinous (MC) carcinomas."</p> <p>21 Correct?</p> <p>22 A Correct.</p> <p>23 Q Okay. And then if we look back at page</p> <p>24 2 of Nunes, in the second sentence of the first</p>
<p style="text-align: right;">Page 327</p> <p>1 of the page, you indicate that ovarian cancer is</p> <p>2 the major cause of death from gynecologic disease</p> <p>3 and the second most common gynecologic malignancy</p> <p>4 worldwide; correct?</p> <p>5 A Correct.</p> <p>6 Q And then in your report you cite Nunes</p> <p>7 and Serpa, the article we've just marked as</p> <p>8 Exhibit 21, as well as Siegel and Torre; correct?</p> <p>9 A Yes.</p> <p>10 Q If we look at page 2 of the Nunes</p> <p>11 article, the exact same sentence appears on -- at</p> <p>12 the bottom of page 2 under the heading of</p> <p>13 "Ovarian Cancer, an Overview"; correct?</p> <p>14 A Correct.</p> <p>15 Q Right.</p> <p>16 A That's correct.</p> <p>17 Q Okay. And it's --</p> <p>18 A It's not quite the same sentence, given</p> <p>19 that it's the same initial statement, not an</p> <p>20 identical sentence.</p> <p>21 Q Very close to identical?</p> <p>22 A Well, they -- they both -- they both</p> <p>23 introduce the same facts.</p> <p>24 Q Okay. Then if we go down a little bit</p>	<p style="text-align: right;">Page 329</p> <p>1 paragraph under "Ovarian Cancer, an Overview,"</p> <p>2 the nearly identical sentence appears there.</p> <p>3 Correct?</p> <p>4 MS. O'DELL:</p> <p>5 Object to the form.</p> <p>6 A The two sentences stating the same</p> <p>7 fundamental facts regarding ovarian cancer and</p> <p>8 the histological types are -- yes, I agree.</p> <p>9 MR. FERGUSON:</p> <p>10 Q With almost the same wording.</p> <p>11 MS. O'DELL:</p> <p>12 Object to the form.</p> <p>13 A They have similar wording.</p> <p>14 MR. FERGUSON:</p> <p>15 Q Remarkably similar; correct?</p> <p>16 MS. O'DELL:</p> <p>17 Object to the form.</p> <p>18 A I wouldn't call it -- so they --</p> <p>19 Again, we're stating fundamental basic</p> <p>20 facts around histological type and following a</p> <p>21 number of, again, factual observations for what</p> <p>22 the state of the art for genetic knowledge</p> <p>23 in -- in different genes and different proteins</p> <p>24 is as it relates to our understanding of -- of</p>

83 (Pages 326 to 329)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 330</p> <p>1 cancer with, again, appropriate reference for</p> <p>2 those -- for those studies.</p> <p>3 MR. FERGUSON:</p> <p>4 Q And then if we look at the following</p> <p>5 paragraphs, the first full paragraph there on</p> <p>6 page 6, in your report you have a sentence that</p> <p>7 starts "low grade OSC cases generally have</p> <p>8 genetic alterations" in a number of items you've</p> <p>9 listed; correct?</p> <p>10 A Correct.</p> <p>11 Q Okay. And that sentence ends with the</p> <p>12 words or "p13/Ras/Notch/FOXMI." Correct?</p> <p>13 A Correct.</p> <p>14 Q Okay. And then if we go back to Nunes,</p> <p>15 if you look at that same paragraph we've been</p> <p>16 talking about -- and those -- there's an</p> <p>17 introductory phrase that you don't have, and then</p> <p>18 it starts with "low grade OSC generally</p> <p>19 comprising." Slightly different wording, but you</p> <p>20 list the same types of receptors and the same</p> <p>21 types of items. Correct?</p> <p>22 A Yes. That's providing a review of,</p> <p>23 again, the known associations between specific</p> <p>24 ovarian subtypes and their most commonly referred</p>	<p style="text-align: right;">Page 332</p> <p>1 MS. O'DELL:</p> <p>2 I'm sorry.</p> <p>3 MR. FERGUSON:</p> <p>4 Q -- on page 2.</p> <p>5 A Yes.</p> <p>6 MR. FERGUSON:</p> <p>7 Sorry. Leigh, it's on page -- the</p> <p>8 bottom of page 2.</p> <p>9 MS. O'DELL:</p> <p>10 Oh, I'm there. When you said the top,</p> <p>11 I got --</p> <p>12 MR. FERGUSON:</p> <p>13 No worries. That's -- my mistake.</p> <p>14 Q Okay. It says "EC subtypes," and then</p> <p>15 it goes to mucin-coding genes on the top of page</p> <p>16 3. Correct?</p> <p>17 A Correct.</p> <p>18 Q Again, that paragraph is nearly</p> <p>19 identical to the one in your report. Correct?</p> <p>20 MS. O'DELL:</p> <p>21 Object to the form.</p> <p>22 MR. FERGUSON:</p> <p>23 Q Same word, same order, same citations;</p> <p>24 correct?</p>
<p style="text-align: right;">Page 331</p> <p>1 genetic information or genetic predis- --</p> <p>2 sorry -- mutated genes. So I'm -- that's right.</p> <p>3 Q Okay.</p> <p>4 A They are -- they are similar in that</p> <p>5 both are, again, introducing factual information</p> <p>6 about the current knowledge in ovarian cancer in</p> <p>7 this literature, again pointing out that</p> <p>8 referencing the papers that they both came from,</p> <p>9 being the Nunes as well as the appropriate</p> <p>10 references.</p> <p>11 Q Okay. And, then, the paragraph below</p> <p>12 that starts endo- -- "endometrioid carcinoma,"</p> <p>13 paren, "(EC)." Correct?</p> <p>14 A Correct.</p> <p>15 Q If we look --</p> <p>16 And then that goes all the way to the</p> <p>17 word "mucin-coding genes" with two citations;</p> <p>18 correct?</p> <p>19 A Correct.</p> <p>20 Q If we look at 2 and the top of page 3</p> <p>21 in Nunes, there's a sentence that starts "EC."</p> <p>22 It does not spell out endometrioid carcinoma. Do</p> <p>23 you see that four lines from the top? I'm sorry.</p> <p>24 Four lines from the bottom --</p>	<p style="text-align: right;">Page 333</p> <p>1 MS. O'DELL:</p> <p>2 Object to the form.</p> <p>3 A So my -- my report is similar to the</p> <p>4 review article. It -- it's listing the subtypes</p> <p>5 of ovarian cancer and -- based on the Nunes</p> <p>6 paper, which is a 2018 publication, so a more</p> <p>7 current review. I'm, again, providing that</p> <p>8 referenced information about the -- the -- this</p> <p>9 observation.</p> <p>10 Q You're citing the same references as</p> <p>11 Nunes; correct?</p> <p>12 A Yes.</p> <p>13 Q You cite the -- the various gene --</p> <p>14 expression of gene in the same order they do,</p> <p>15 so --</p> <p>16 Correct?</p> <p>17 A Yes.</p> <p>18 Q And is that just coincidental? That's</p> <p>19 just happened? You happened to have put this</p> <p>20 paragraph in the same order with the same</p> <p>21 notations as -- as Nunes?</p> <p>22 MS. O'DELL:</p> <p>23 Object to the form.</p> <p>24 A Well, I'm listing the same information</p>

84 (Pages 330 to 333)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 334</p> <p>1 that's contained in the Nunes paper. And seeing</p> <p>2 as that -- this was a review of the literature</p> <p>3 with -- you know, based on the state of the art,</p> <p>4 the Nunes review is exactly that. And, again,</p> <p>5 I'm -- I'm repeating the information regarding</p> <p>6 the specific gene information as it relates to</p> <p>7 this -- this ovarian cancer risk and -- and --</p> <p>8 and, again, appropriately citing the basic</p> <p>9 studies as Nunes did.</p> <p>10 MR. FERGUSON:</p> <p>11 Q With virtually the same wording?</p> <p>12 A With similar wording, yes.</p> <p>13 Q Let's look at page -- page 7.</p> <p>14 MS. O'DELL:</p> <p>15 His report?</p> <p>16 MR. FERGUSON:</p> <p>17 Q Yeah. I apologize. Your report.</p> <p>18 We can set Nunes aside now.</p> <p>19 You have a paragraph starts -- that</p> <p>20 starts "individuals can inherit mutations in</p> <p>21 BRCA1, BRCA2 or p53."</p> <p>22 See it?</p> <p>23 A Uh-huh.</p> <p>24 Q And you say, "These defects allow</p>	<p style="text-align: right;">Page 336</p> <p>1 or p53 mutations can be considered causes of</p> <p>2 cancer?</p> <p>3 MS. O'DELL:</p> <p>4 Object to the form.</p> <p>5 A No. Not -- not specifically causal. I</p> <p>6 think the -- each of these -- as we've discussed,</p> <p>7 each of these genes, BRCA1 and BRCA2, or starting</p> <p>8 with BRCA1 and BRCA2, increase the probability of</p> <p>9 a -- of a person -- generally women -- getting</p> <p>10 breast or ovarian cancer but do not exclusively</p> <p>11 mean somebody with that mutation will get cancer.</p> <p>12 So, with that knowledge, I would not</p> <p>13 consider BRCA1 and BRCA2 mutation alone</p> <p>14 sufficient to cause cancer. It increased the</p> <p>15 risk.</p> <p>16 And, as we talked about, p53 is a bit</p> <p>17 more of a higher-risk gene, and the question as</p> <p>18 to whether or not it is possible for someone to</p> <p>19 have a -- what the rate of someone having a p53</p> <p>20 mutation and not getting cancer, I believe, is</p> <p>21 currently unknown. But there, again, is a much</p> <p>22 higher probability of developing -- developing</p> <p>23 cancer.</p> <p>24 MR. FERGUSON:</p>
<p style="text-align: right;">Page 335</p> <p>1 additional mutations to accumulate in cells and</p> <p>2 lead to a higher probability of cells being</p> <p>3 cancerous."</p> <p>4 Correct?</p> <p>5 A Correct.</p> <p>6 Q And you've indicated earlier in your</p> <p>7 report that cancer is caused by mutations.</p> <p>8 Correct?</p> <p>9 A Correct.</p> <p>10 Q And you say here that mutations in</p> <p>11 BRCA1, BRCA2 or p53 can result in the</p> <p>12 accumulation of additional mutations in cells.</p> <p>13 Correct?</p> <p>14 MS. O'DELL:</p> <p>15 Object to the form.</p> <p>16 A Yeah. I made the statement that BRCA1,</p> <p>17 BRCA2 and p53, they can be inherited and then, in</p> <p>18 turn, positive for those gene mutations.</p> <p>19 MR. FERGUSON:</p> <p>20 Q Okay. Would you --</p> <p>21 A So I guess if you could ask the</p> <p>22 question again to make sure I understand it.</p> <p>23 Q Well, let me -- doesn't this paragraph</p> <p>24 mean, in your comments here, that BRCA1, BRCA2,</p>	<p style="text-align: right;">Page 337</p> <p>1 Q And then the last line there of page 7,</p> <p>2 you say, "The lifetime risk for ovarian cancer is</p> <p>3 approximately 40 percent for BRCA1 carriers and</p> <p>4 15 to 20 percent for BRCA2 carriers."</p> <p>5 Correct?</p> <p>6 A Correct. Based on -- based on the</p> <p>7 study that I referenced, yes.</p> <p>8 Q Right.</p> <p>9 And -- and the -- the -- if we look at</p> <p>10 the increased risk of 40 percent as compared to</p> <p>11 the risk of cancer in the -- of ovarian cancer in</p> <p>12 the general population, that's a 25-fold increase</p> <p>13 for BRCA1 and about a 7- or 8-fold increase for</p> <p>14 BRCA2; correct?</p> <p>15 MS. O'DELL:</p> <p>16 Object to the form.</p> <p>17 A I -- I would have to -- to determine</p> <p>18 that. But I would say so. I'm certainly</p> <p>19 comfortable stating that the lifetime risk for</p> <p>20 ovarian cancer is approximately 40 percent. I'd</p> <p>21 have to verify your -- your math about that</p> <p>22 indicating a 25-fold increase.</p> <p>23 MR. FERGUSON:</p> <p>24 Q Do you know what the rate in the</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 338</p> <p>1 general population of ovarian cancer is?</p> <p>2 A It's fairly low. If I -- thinking of</p> <p>3 the cohort studies that were reviewed as part of</p> <p>4 this, it was roughly a hundred to 200 cases per</p> <p>5 30- to 40,000 women in those -- in those studies,</p> <p>6 so relatively low.</p> <p>7 Q And if we go to the top of the next</p> <p>8 page, you say -- it's page 8 -- "Therefore, the</p> <p>9 presence of mutations in the BRCA genes do not</p> <p>10 guarantee that carriers will get cancer. The</p> <p>11 presence of these mutations increases a person's</p> <p>12 risk of developing cancer when exposed to a</p> <p>13 carcinogen."</p> <p>14 Correct?</p> <p>15 A Correct.</p> <p>16 Q And you cite Park, Vitonis, and Wu for</p> <p>17 that. Is that correct?</p> <p>18 A That's correct.</p> <p>19 Q Looking at Park, isn't it true that</p> <p>20 Park does not supply any evidence to support your</p> <p>21 claim that mutations in BRCA1, BRCA2 and/or p53</p> <p>22 increase a person's risk of developing cancer</p> <p>23 when exposed to a carcinogen?</p> <p>24 A I'd have to remind myself of what's in</p>	<p style="text-align: right;">Page 340</p> <p>1 So the -- the Park paper does discuss</p> <p>2 the relationship of ovarian cancer risk relative</p> <p>3 to benign gynecological conditions.</p> <p>4 Q And -- and your comment that you've</p> <p>5 cited these studies for is the presence of these</p> <p>6 mutations increases a person's risk of developing</p> <p>7 cancer when exposed to a carcinogen. And these</p> <p>8 mutations would be what you've been talking about</p> <p>9 in this paragraph, the B -- the BRCA1, BRCA2, and</p> <p>10 p53; correct?</p> <p>11 MS. O'DELL:</p> <p>12 Object to the form.</p> <p>13 A The sentence is worded, "The presence</p> <p>14 of these mutations increases a person's risk of</p> <p>15 developing cancer when exposed to a carcinogen."</p> <p>16 MR. FERGUSON:</p> <p>17 Q Right. Right.</p> <p>18 And, for example, in Vitonis, isn't it</p> <p>19 true that BRCA1, BRCA2 and p53 were not even</p> <p>20 determined in that study and, instead, Jewish</p> <p>21 ethnicity was used as a surrogate for a woman's</p> <p>22 risk of having a mutation in one of these genes?</p> <p>23 Do you recall that --</p> <p>24 A Again, I would have --</p>
<p style="text-align: right;">Page 339</p> <p>1 Park.</p> <p>2 Q Are you going through the entirety of</p> <p>3 the article?</p> <p>4 A I'm just reminding myself the content</p> <p>5 to see if I could find something that was</p> <p>6 specifically related to your question about the</p> <p>7 presence of a BRCA1 or 2 mutation.</p> <p>8 Q Okay. Is the BRCA1, BRCA2, p53, any of</p> <p>9 those even mentioned in the article?</p> <p>10 And -- and I'm not sure we'll have time</p> <p>11 for you to go through each one of them in this</p> <p>12 much --</p> <p>13 You've got -- you cited them for these</p> <p>14 propositions. I'm trying to ask you why you</p> <p>15 cited them for this proposition.</p> <p>16 A I -- I'd have to look in more detail.</p> <p>17 I don't have a specific answer regarding the --</p> <p>18 regarding BRCA1 --</p> <p>19 Q Okay.</p> <p>20 A -- I'm sorry -- BRCA genes.</p> <p>21 I would suspect the Park reference was</p> <p>22 more in the discussion of overall relative risk</p> <p>23 of developing cancer and not necessarily</p> <p>24 exclusive to the presence of a mutation.</p>	<p style="text-align: right;">Page 341</p> <p>1 Q -- one way or the other?</p> <p>2 MS. O'DELL:</p> <p>3 Objection.</p> <p>4 A I would have to review the -- review</p> <p>5 the paper. Because part of the review is to</p> <p>6 be -- include appropriate references with regards</p> <p>7 to ovarian cancer risk, and those may -- I think</p> <p>8 those publications provide some information in</p> <p>9 that space.</p> <p>10 MR. FERGUSON:</p> <p>11 Q All right. But when you cite studies</p> <p>12 for a statement in your report, shouldn't the</p> <p>13 studies relate to that statement?</p> <p>14 MS. O'DELL:</p> <p>15 Object to the form.</p> <p>16 A Well, the studies relate to a person's</p> <p>17 risk of developing cancer. But I -- I think</p> <p>18 it -- it doesn't change the accuracy of the</p> <p>19 presence of the mutation relative to that risk.</p> <p>20 But the -- I don't have a -- a good answer as far</p> <p>21 as relationship of BRCA1 and 2 to the Park paper.</p> <p>22 MR. FERGUSON:</p> <p>23 Q And -- and, then --</p> <p>24 Well, we talked about Vitonis, too.</p>

86 (Pages 338 to 341)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 342</p> <p>1 And then let's get to Wu. 2 MS. O'DELL: 3 Object to the form. You didn't comment 4 specifically about Vitonis, if you've got an 5 issue with Vitonis. You know, it's not fair to 6 assume that because I don't think you asked a 7 direct question. 8 MR. FERGUSON: 9 Okay. I thought I did, but I could be 10 mistaken. 11 MS. O'DELL: 12 You mentioned it, but I don't think 13 you -- I think it was more you rather than asking 14 a question. 15 MR. FERGUSON: 16 Q With regard to Wu, do you recall that, 17 in Wu, BRCA1, BRCA2, and p53 inherited carrier 18 mutation status were not even determined in that 19 study? Do you recall that -- 20 A The -- 21 Q -- one way or the other? 22 MS. O'DELL: 23 Object to the form. 24 A The Wu paper specifically discussed</p>	<p style="text-align: right;">Page 344</p> <p>1 syndrome patients have an increased risk of 2 cancer when exposed to a carcinogen. Correct? 3 A Correct. 4 Q What carcinogens are you referring to? 5 A I'm not -- not referring to a specific 6 carcinogen. I'm using the term "carcinogen" to 7 refer to an insult that would result in DNA 8 damage specifically because, similar to the BRCA 9 mutations, Lynch syndrome impairs DNA mismatch 10 repair. 11 So that defect alone is not sufficient 12 to result in a cellular transformation, so 13 something else has to occur. And when we 14 consider that carcinogens are -- the term 15 "carcinogen" generally refers to something that 16 has the potential to damage cellular components 17 or DNA, it's putting the -- 18 Inability to repair along with the 19 presence of a carcinogen is where that sentence 20 comes from. 21 Q So -- and I want to make sure I 22 understand what you're saying. Are you saying 23 that Lynch syndrome patients have an increased 24 risk of developing cancer after exposure to a</p>
<p style="text-align: right;">Page 343</p> <p>1 nongenetic risk factors. 2 MR. FERGUSON: 3 Q Let's go to the next paragraph, and 4 there you talk about single nucleotide variance, 5 SNVs; correct? 6 A Towards the bottom of the paragraph. 7 As -- in terms of modifiers, yes. 8 Q Yeah. Are -- are single nucleotide 9 variants mutations? 10 A Yes. 11 Q Do most SNVs result in functionally 12 defective proteins? 13 A Statistically speaking on a genome-wide 14 basis, no. 15 So a -- a single nucleotide variant is 16 a variant at any point. And if we consider 17 statistically that about 1 percent of the genome 18 encodes proteins, again, it's statistically less 19 likely that any SNV would affect a protein. 20 Q Okay. Let's look at the next 21 paragraph. There you talk about Lynch syndrome; 22 correct? 23 A Correct. 24 Q And you make a statement that Lynch</p>	<p style="text-align: right;">Page 345</p> <p>1 carcinogen, just like everyone else? 2 A No. I'm stating that Lynch syndrome -- 3 MS. O'DELL: 4 Object to the form. Excuse me. 5 A Lynch syndrome is a hereditary 6 condition that increases the overall risk of 7 cancer to an individual, similar to BRCA1 and 2 8 mutation. 9 MR. FERGUSON: 10 Q So you -- are you claiming that Lynch 11 syndrome patients have a greater increase in 12 relative risk when exposed to a particular 13 carcinogen than do people without Lynch syndrome? 14 MS. O'DELL: 15 Object to the form. 16 A No, I'm not making that statement, to a 17 specific carcinogen. 18 MR. FERGUSON: 19 Q In your next paragraph you talk of -- 20 you start with "Myriad Genetics," and you say, 21 "As with all inherited traits, a positive family 22 history is the strongest indicator of the 23 presence of genetic risk alleles in an 24 individual."</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 346</p> <p>1 Correct?</p> <p>2 A Correct.</p> <p>3 Q Isn't it true that many women who have</p> <p>4 inherited mutations like BRCA1 or BRCA2 and genes</p> <p>5 that predispose to ovarian cancer development do</p> <p>6 not have a family history of breast or ovarian</p> <p>7 cancer?</p> <p>8 A So the -- your -- your question is a</p> <p>9 little bit different than the statement. So</p> <p>10 the -- if I could clarify the statement in the</p> <p>11 report, it is more that a positive family history</p> <p>12 would be a likely indicator that someone has a</p> <p>13 genetic risk variant such as BRCA1 and 2.</p> <p>14 Q Isn't it true that family history is</p> <p>15 not a sensitive or specific indicator of</p> <p>16 whether -- of whether a particular woman has</p> <p>17 inherited a mutation in a gene associated with</p> <p>18 increased risk of ovarian cancer?</p> <p>19 MS. O'DELL:</p> <p>20 Object to the form.</p> <p>21 A I would say that family -- I would ask</p> <p>22 to define "sensitive" or "specific," because in</p> <p>23 genetics overall, family history remains a</p> <p>24 valuable and important characteristic in terms of</p>	<p style="text-align: right;">Page 348</p> <p>1 number higher than that if you're looking at</p> <p>2 indirect or genetic complex formation.</p> <p>3 You know, depends how far down the</p> <p>4 cellular control and signal transduction and</p> <p>5 growth and proliferation road that we go as far</p> <p>6 as how many genes. But I'm sure, as everyone</p> <p>7 well appreciates, everything in biology is</p> <p>8 interrelated in some form.</p> <p>9 And, so, it -- but I would say this</p> <p>10 statement here is that our ability to look at</p> <p>11 large-scale genetic analysis in individuals of a</p> <p>12 variety of cancer types, given the number of</p> <p>13 individuals affected by cancer and the analysis</p> <p>14 of their genetics, we've been able to identify</p> <p>15 many of -- many of the fundamental or most --</p> <p>16 perhaps most of the fundamental genes involved in</p> <p>17 that initial disease initiation or progression.</p> <p>18 It's important that it is not a</p> <p>19 comprehensive list. Hence, it is not "all," but</p> <p>20 there are a large number of genes that are well</p> <p>21 established.</p> <p>22 Q Okay. Let's look at the next page, 10.</p> <p>23 And you have a paragraph that starts</p> <p>24 "Macrophages."</p>
<p style="text-align: right;">Page 347</p> <p>1 determining the genetic component of -- of any</p> <p>2 disease, cancer included. And, so, if there's</p> <p>3 something exact regarding its sensitivity or</p> <p>4 specificity that I can comment on, I will if I</p> <p>5 know the answer. But...</p> <p>6 MR. FERGUSON:</p> <p>7 Q In -- in the top of the page -- of</p> <p>8 page 9, the next page, you indicate, "Because of</p> <p>9 the large number of individuals tested and the</p> <p>10 ability to trace their genetic inheritance, the</p> <p>11 genes involved in cancer development are well</p> <p>12 established."</p> <p>13 Is that correct?</p> <p>14 A Correct. That's what I state. I did</p> <p>15 make that statement.</p> <p>16 Q And given that they're well</p> <p>17 established, can you name all of the inherited</p> <p>18 genes that have been identified as being</p> <p>19 associated with an increased risk of ovarian</p> <p>20 cancer?</p> <p>21 A No, not -- I can't name them all off</p> <p>22 the top of my head, no. There's something in the</p> <p>23 neighborhood of 500 to -- 500 genes of strong</p> <p>24 association of cancer risk and progression, some</p>	<p style="text-align: right;">Page 349</p> <p>1 A Uh-huh.</p> <p>2 Q And the last sentence says, "Generally</p> <p>3 speaking, macrophages can increase inflammation</p> <p>4 or decrease inflammation, depending on the</p> <p>5 cytokines released."</p> <p>6 Correct?</p> <p>7 A Correct.</p> <p>8 Q So, with that statement, do you agree</p> <p>9 that inflammation can have both protumorigenic</p> <p>10 and antitumorigenic effects, depending on</p> <p>11 context, just as you state here for macrophages?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form.</p> <p>14 A No, I -- I would not agree with that.</p> <p>15 I -- I don't know of any evidence of that, that</p> <p>16 inflammation, as a physiological phenomenon, acts</p> <p>17 as an antitumor effect.</p> <p>18 MR. FERGUSON:</p> <p>19 Q Going to the next page, the page 11 --</p> <p>20 I'm trying to get through this</p> <p>21 hopefully within the next 15 minutes.</p> <p>22 -- under the role of inflammation in</p> <p>23 ovarian cancer --</p> <p>24 Are you with me there?</p>

88 (Pages 346 to 349)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 350</p> <p>1 A I am.</p> <p>2 Q And you're obviously talking about the</p> <p>3 role of inflammation there. Isn't it true that</p> <p>4 no published animal model has ever shown that</p> <p>5 inducing inflammation induces the development of</p> <p>6 ovarian cancer?</p> <p>7 MS. O'DELL:</p> <p>8 Object to the form.</p> <p>9 A We've been -- earlier today we were</p> <p>10 discussing some animal models as it relates to --</p> <p>11 MR. FERGUSON:</p> <p>12 Q Yeah. You and Miss Brown talked about</p> <p>13 a number of animal models.</p> <p>14 A Yeah.</p> <p>15 Q And -- and what I'm trying to ask you,</p> <p>16 is there any of those animal models or any others</p> <p>17 that have ever shown that inducing inflammation</p> <p>18 induces the development of ovarian cancer?</p> <p>19 A I didn't -- I didn't look specifically</p> <p>20 for an animal study of that type in the process</p> <p>21 of developing the report.</p> <p>22 Q Later down that page, you talk about</p> <p>23 two models. "The literature reviews as well as</p> <p>24 many direct studies feature the immune system as</p>	<p style="text-align: right;">Page 352</p> <p>1 anything on that, so that's -- that's fine.</p> <p>2 Let's move on.</p> <p>3 A Okay.</p> <p>4 Q I think you've stated earlier that your</p> <p>5 opinion in this case is based on the totality of</p> <p>6 what is included in the product, the talcum</p> <p>7 powder products. Is that correct?</p> <p>8 A Correct.</p> <p>9 Q So you're -- you cannot distinguish</p> <p>10 the -- the carcinogenicity of the constituent</p> <p>11 parts of the talcum powder products, correct,</p> <p>12 including the fragrance?</p> <p>13 MS. O'DELL:</p> <p>14 Object to the form.</p> <p>15 A I -- I was -- I was not asked to -- to</p> <p>16 provide that delineation. And, so, instead,</p> <p>17 subsequent to seeing some of the other expert</p> <p>18 reports, we began with talcum powder as a product</p> <p>19 and then have since learned more about the</p> <p>20 constituent components, including asbestos,</p> <p>21 fragrance, potential for heavy metals, which I</p> <p>22 understand or I've observed that there's a</p> <p>23 variety of testing documents that -- that show a</p> <p>24 variety of results.</p>
<p style="text-align: right;">Page 351</p> <p>1 being an important mediator of ovarian</p> <p>2 carcinogenesis via two models, chronic</p> <p>3 inflammation and incessant ovulation."</p> <p>4 Correct?</p> <p>5 A Correct.</p> <p>6 Q Is it your opinion that incessant</p> <p>7 ovulation is a form of chronic inflammation?</p> <p>8 A It is not.</p> <p>9 Q Isn't it true that there's no</p> <p>10 pathological evidence in humans that perineal</p> <p>11 talc users have ovarian inflammation?</p> <p>12 MS. O'DELL:</p> <p>13 Object to the form.</p> <p>14 A I'm thinking.</p> <p>15 I would have to review the --</p> <p>16 I'm sorry. That's -- it's --</p> <p>17 MR. FERGUSON:</p> <p>18 Q Okay.</p> <p>19 A I would -- again, I would have to look</p> <p>20 more carefully for that. I can't -- I can't name</p> <p>21 a study of that type right now.</p> <p>22 Q So I think you've said previously --</p> <p>23 Are you done looking?</p> <p>24 I understood you couldn't give me</p>	<p style="text-align: right;">Page 353</p> <p>1 So, to answer your question, I did not</p> <p>2 specifically evaluate the individual specific</p> <p>3 components in any -- in any individual product as</p> <p>4 it relates. Instead, remained focused on the</p> <p>5 mechanism for the complete -- complete product.</p> <p>6 MR. FERGUSON:</p> <p>7 Q And you've made reference to heavy</p> <p>8 metals throughout your testimony on occasion. Do</p> <p>9 you recall that?</p> <p>10 A I do.</p> <p>11 Q Do you have any opinions that any of</p> <p>12 these heavy metals contribute to the inflammation</p> <p>13 process that you've been talking about?</p> <p>14 A The -- to the inflammation --</p> <p>15 I'm not aware of any direct evidence</p> <p>16 for heavy metal contribution to the inflammation</p> <p>17 process that we've been discussing. Instead, the</p> <p>18 heavy metals, particularly chromium, caught my</p> <p>19 attention because of its well-established ability</p> <p>20 to directly damage DNA and, therefore, you know,</p> <p>21 potentially play a role in carcinogenesis.</p> <p>22 Q Do you have any knowledge or opinion</p> <p>23 about how much chromium you claim is in the -- in</p> <p>24 the body powder products?</p>

89 (Pages 350 to 353)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 354</p> <p>1 MS. O'DELL: 2 Object to the form. 3 A I wasn't asked to evaluate the amount 4 of chromium or whether it was sufficient for 5 damage. It was more reviewing. I would have to 6 defer to other experts who have done the testing 7 on the products. 8 MR. FERGUSON: 9 Q So you have no opinion on that? 10 MS. O'DELL: 11 Object to the form. 12 A I'm sorry. An opinion on the amount of 13 chromium? 14 MR. FERGUSON: 15 Q Correct. 16 A Again, I wasn't asked to generate such 17 an opinion. 18 Q I think -- I think I'm almost done. 19 Isn't it true that published data have 20 demonstrated that talc is not genotoxic and does 21 not cause mutations? 22 MS. O'DELL: 23 Object to the form. 24 A I'm not aware of a study that</p>	<p style="text-align: right;">Page 356</p> <p>1 talc with asbestiform bodies, I think would be 2 very reasonable to state that it has mutagenic 3 properties. 4 MR. FERGUSON: 5 Q And can you cite me any literature for 6 that? 7 A I would simply refer to the -- much of 8 the body of asbestos literature for the -- for 9 that. 10 MR. FERGUSON: 11 I think that's all I have. I'll turn 12 it over to someone else to ask some questions. 13 MS. BROWN: 14 Anybody with some more? 15 MS. O'DELL: 16 I'm going to take a break for a few 17 minutes. 18 VIDEOGRAPHER: 19 Going off the record. The time is 20 4:54 p m. 21 (OFF THE RECORD.) 22 VIDEOGRAPHER: 23 We're back on the record. The time is 24 5:20 p m.</p>
<p style="text-align: right;">Page 355</p> <p>1 specifically looked at the genotoxicity of -- of 2 talc. And I think it would certainly warrant 3 defining which type of talc and components 4 therein. But I'm -- I'm not aware of a study 5 that has concluded that there are no genotoxic 6 effects of any type of talc. 7 MR. FERGUSON: 8 Q Would you agree there's no evidence 9 that talc causes sister chromatid exchange or 10 unscheduled DNA synthesis? 11 MS. O'DELL: 12 Object to the form. 13 A I didn't -- I didn't review the 14 literature for those two specific phenomenon. I 15 would have to, again, specifically look or review 16 for that. 17 MR. FERGUSON: 18 Q So, as you sit here, you have no 19 opinion as to whether talc is or is not 20 mutagenic? 21 MS. O'DELL: 22 Object to the form. 23 A No. We've -- so talc in general, 24 particularly in its -- in its form of fibrous</p>	<p style="text-align: right;">Page 357</p> <p>1 EXAMINATION 2 BY MS. O'DELL: 3 Q Dr. Levy, I have just a few follow-up 4 questions for you. 5 I'm gonna ask you to turn to page 14 of 6 your report. 7 And earlier today -- 8 I'm going to ask, Doctor, if you could 9 put the exhibits in front of you, and we'll pull 10 those out. 11 But earlier today you were asked about 12 a letter from the FDA that was marked as Exhibit 13 Number 16, and if you could pull that out of your 14 stack there. And, specifically, if you'll turn 15 to page 4 of the letter. 16 And you'll recall that this letter was 17 written in 2014. Do you remember that? 18 A Yes. 19 Q And if you look, however, at page 4 of 20 the letter, it appears that the FDA's review of 21 the relevant toxicity literature stopped at the 22 year 2008. Fair? 23 MS. BROWN: 24 Objection to the form.</p>

90 (Pages 354 to 357)

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 358</p> <p>1 MS. O'DELL: 2 Q Did the FDA's review of the toxicity 3 literature stop in 2008? 4 A Yes. 5 Q And if you look at page 14 of -- of 6 your report, your review of the literature 7 included multiple references that were published 8 after 2008? 9 MS. BROWN: 10 Form. 11 A That's correct. 12 MS. O'DELL: 13 Q And, in fact, you cited Shukla that was 14 published in -- 15 Was Shukla published in 2009? 16 A Yes. The reference is in the report to 17 2009. 18 Q Yes. 19 And, in addition to that, did you cite 20 other references in support of your opinion that 21 talc powder causes inflammation that were dated 22 and published after 2008? 23 A I did. 24 Q And, so, the suggestion by counsel for</p>	<p style="text-align: right;">Page 360</p> <p>1 Objection to the form of the question. 2 A Yes, we -- we had a discussion 3 regarding the results shown in Figure 3, the 4 level of exposure of talc as well as its 5 duration. Sorry. The talc dose as well as 6 duration. 7 MS. O'DELL: 8 Q And in the -- if you'll look at 9 Figure 1, Doctor, explain to us, please, what 10 Figure 1 describes in terms of the viability of 11 the cells at the 72-hour mark. 12 A So the -- so Figure 1 is a graph 13 describing percent cell viability versus the 14 different normal or variant cells at a 24-hour 15 and 72-hour time point, two different ovarian 16 cancer cell lines, as well as doses of talc from 17 zero micrograms per milliliter up to 500 18 micrograms per milliliter, and each of those is 19 applied. 20 And at the 72-hour time point in both 21 cell lines, OSE2a and GCA1 -- GC1a shows a 22 decrease in cellular viability that is 23 dose-dependent in each of the four cell lines. 24 Q Okay. And --</p>
<p style="text-align: right;">Page 359</p> <p>1 Johnson & Johnson that somehow the FDA had 2 reviewed the literature for toxicity up until the 3 date of this letter would have been incorrect? 4 MS. BROWN: 5 Objection to the form of the question. 6 A As -- as we discussed, the -- the 7 letter from the FDA dated April 1st, 2014, states 8 to include literature from 1980 to 2008. 9 MS. O'DELL: 10 Q Let me ask you -- 11 You can put that aside, Dr. Levy. 12 Thank you. 13 And I want to ask you to pull out of 14 the stack the Exhibit 17, which is the Buz'Zard 15 paper. 16 A I have it. 17 Q And if you'll turn to page 581. 18 A Okay. 19 Q And just to orient our discussion, 20 counsel for Johnson & Johnson suggested that -- 21 that this paper showed a decrease in reaction or 22 reactive oxygen species at the longest time 23 interval. Do you recall that discussion? 24 MS. BROWN:</p>	<p style="text-align: right;">Page 361</p> <p>1 A Sorry. Each of the two cell lines. 2 Q And is it fair to say that the reason 3 you don't see dose response, you know, at the -- 4 at the greatest magnitude is because the cells 5 essentially die? 6 MS. BROWN: 7 Objection to the form. 8 A Well, I would say if we consider the 9 results displayed in Figure 1 in relation to the 10 results displayed in Figure 3, an ex- -- an 11 explanation for the concentrating on the 500 -- 12 the highest dose, the 500 micrograms per 13 milliliter, in the talc exposure, the decrease in 14 cellular viability is an -- is an explanation -- 15 could be an explanation for the decrease in 16 reactive oxygen species. 17 MS. O'DELL: 18 Q Okay. Thank you, Doctor. 19 And if you'll put that aside and turn 20 to Exhibit 7, which was the Hamilton paper we 21 spent quite a lot of time on earlier. 22 Do you recall the -- that discussion 23 regarding the Hamilton paper? 24 A I do.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 362</p> <p>1 Q And what was the purpose for which you 2 cited the Hamilton paper? 3 A That it was one of the available animal 4 studies looking at the effects of talc on a rat 5 ovary. 6 Q And did the paper show that there was a 7 increase in inflammation as result of talc? 8 A Yes, in the form of foreign body 9 granulomas observed in five of the injected 10 ovaries. 11 Q And you're looking at, I guess, that 12 last sentence on page 103 and carrying over to 13 the -- to the narrative on page 105? 14 A Cellular foreign body? 15 Q Yes. 16 A Foreign body granulomas without any 17 surrounding inflammation were seen in five of the 18 injected ovaries. And similar lesions were not 19 uncommonly noted in the supracapsular fat in the 20 connective tissue matrix of the capsule. 21 Q And if you'll look down in the 22 discussion section, Dr. Levy, the first paragraph 23 there in your -- where -- beginning 24 "Unfortunately," does it appear that talc also</p>	<p style="text-align: right;">Page 364</p> <p>1 principle been published in the peer-reviewed 2 literature? 3 A It has. 4 Q And, in regard to ovarian cancer, prior 5 to becoming involved in the litigation, did you 6 hold the opinion that inflammation was a part of 7 the development of ovarian cancer? 8 A Yes. 9 Q And has that been researched and that 10 research published in the peer-reviewed 11 literature? 12 A It has. 13 Q In the same way, has the fact that 14 talc, talcum powder, induces inflammation been 15 published in the peer-reviewed literature? 16 MS. BROWN: 17 Objection to the form. 18 A Yes. 19 MS. O'DELL: 20 Q And you were asked whether there was 21 evidence that talc caused inflammation in humans. 22 Do you recall that question? 23 A I do. 24 Q And based on your exhaustive review of</p>
<p style="text-align: right;">Page 363</p> <p>1 induced fibrosis -- 2 MS. BROWN: 3 Objection to form. 4 MS. O'DELL: 5 Q -- in the rats? 6 A The manuscript makes the statement 7 that, "Unfortunately, bursal distention occurred 8 as an unforeseen complication" and further states 9 that this probably resulted from talc-induced 10 fibrosis and obliteration of the small channel 11 which normally allows communication between the 12 cavity where the ovary lies and the perineum. 13 Q And though the authors concluded that 14 neoplastic changes were not seen, the authors did 15 find evidence of inflammation in their study? 16 A That's correct. 17 Q Prior to becoming involved in the 18 litigation, Dr. Levy, did you hold the opinion 19 that inflammation is a cause of cancer? 20 A As -- as we've discussed earlier, I 21 certainly held the opinion that, you know, 22 inflammation is a significant and necessary 23 component of cancer progression. 24 Q And has that been -- that general</p>	<p style="text-align: right;">Page 365</p> <p>1 the literature, what evidence would you point to 2 undergirding your opinion that talc causes 3 inflammation in humans? 4 A I think considering the molecular 5 mechanism we were discussing of the recent paper 6 by Saed, et al., again, that we discussed earlier 7 today is a fairly in-depth set of experiments to 8 examine the specific inflammatory response 9 of -- of human cells to -- to talcum powder. 10 Q In addition to the Saed publications, 11 would you -- would you include the Shukla 2009 12 paper in your consideration of talc causing 13 inflammation in humans? 14 A Yes. 15 MS. BROWN: 16 Form. 17 MS. O'DELL: 18 Q You were asked about your methodology 19 numerous times today, and can -- would you 20 describe in -- in general the methodology you 21 have used in reaching your opinions in this case? 22 A Yes. To clarify or perhaps expand on 23 the earlier discussions, my methodology involved 24 a literature review to examine the totality of</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 366</p> <p>1 the information available to the role that talcum 2 powder plays in inflammation in ovarian cancer. 3 And, so, that methodology involved, 4 first, a review of the literature and then a 5 development of a report and then a synthesis of a 6 biologically plausible mechanism where the basis 7 of that plausibility was to ask if each of the 8 different component steps that are described in 9 that mechanism was supported by peer-reviewed 10 research. First, does talc cause inflammation? 11 Second, does inflammation cause cancer? And 12 then, third -- or does inflammation cause ovarian 13 cancer? And then, third, is there -- is that 14 supportive of a overall mechanism of cancer 15 progression and metastasis? 16 Q Can that methodology be replicated? 17 A Certainly. I think, you know, anyone 18 with a similar -- similar background and 19 experience who -- who undertook the same 20 activities would likely -- certainly likely come 21 up with the same -- same conclusions. 22 Q Did you rely on the IARC monograph in 23 relation to nickel, chromium, and cobalt in 24 reaching your opinions in this case?</p>	<p style="text-align: right;">Page 368</p> <p>1 Q Is this the Park paper that you 2 referenced -- 3 MS. BROWN: 4 Counsel, do you have a copy for us? 5 MS. O'DELL: 6 I don't. I'm assuming -- I don't think 7 Ken marked it, but I'm assuming he has a copy. 8 Q Is that the Park paper that you 9 referenced in your report, Dr. Levy? 10 A It is. 11 Q And if you'll turn to page 8 of the 12 paper, about midway down the first column, maybe 13 a little bit less, see the paragraph starting "We 14 did find an association"? Page 8. 15 A I'm looking for the page number. 16 Q Sorry. Let me give you a page number. 17 I'm not sure it has a page number. 18 A No, it doesn't. 19 Q Do you see the paragraph beginning "We 20 did find associations between overall cancer and 21 history of fibroid or ovarian cysts"? Do you see 22 that paragraph? 23 A Well, actually -- yes, I see that 24 paragraph.</p>
<p style="text-align: right;">Page 367</p> <p>1 MS. BROWN: 2 Objection to the form. 3 A I -- so the -- the number of IARC 4 publications were certainly in the material that 5 was reviewed for -- for my -- for my report. 6 MS. O'DELL: 7 Q Based on your review of the literature, 8 is it your opinion that nickel causes 9 inflammation? 10 A Yes. The IARC -- the -- the 11 characterization of those compounds, nickel as 12 well as chromium, among others, are -- would have 13 an inflammatory response. 14 Q You were asked questions earlier 15 today -- actually, not so much earlier -- a few 16 minutes ago regarding the Park paper. And you 17 cited the Park paper on page -- I think it was 8 18 of your report. 19 A Yes. 20 Q And let me show you what I'm marking as 21 Exhibit 22 to your deposition. 22 (DEPOSITION EXHIBIT NUMBER 22 23 WAS MARKED FOR IDENTIFICATION.) 24 MS. O'DELL:</p>	<p style="text-align: right;">Page 369</p> <p>1 Q If you'll look further, the sentence 2 beginning "This observation may suggest," do you 3 see that? 4 A Yes. Uh-huh. 5 Q And the paper says, "This observation 6 may suggest a possible additive or synergistic 7 effect on tumor- -- tumorigenesis influenced by 8 the proinflammatory milieu from an increased 9 burden in the number of benign conditions. 10 Increased risk of serous cancer, ovarian cancer, 11 women with other proinflammatory risk factors has 12 been reported -- reported, most notably in talc 13 users." 14 Do you see that? 15 A I do. 16 Q Is that the section you were thinking 17 of when you cited it in your report? 18 MS. BROWN: 19 Objection to the form. 20 A Yes, it is. 21 MS. O'DELL: 22 Q Let me ask you to -- a couple of other 23 final questions, Dr. Levy. 24 Excuse me. Give me one moment.</p>

Shawn Levy, Ph.D.

Page 370	Page 372
<p>1 In regard to opinions in relation to</p> <p>2 the pathology of ovarian tissue, would you defer</p> <p>3 to a gynecologist or gynecologic oncologist or a</p> <p>4 pathologist regarding that matter?</p> <p>5 A Yes, of course.</p> <p>6 Q You testified earlier today that you</p> <p>7 relied on the Longo testing in -- in reaching</p> <p>8 your opinions in this case.</p> <p>9 MS. BROWN:</p> <p>10 Objection to the form.</p> <p>11 MS. O'DELL:</p> <p>12 Q Did you rely on Dr. Longo's testing</p> <p>13 in -- in reaching your opinions in this case?</p> <p>14 A Yes. They were -- they were one of</p> <p>15 the -- among many of the manuscripts we've been</p> <p>16 discussing.</p> <p>17 Q Yeah.</p> <p>18 In fact, you cite Dr. Longo's report on</p> <p>19 page 15 of your report. Is that right?</p> <p>20 MS. BROWN:</p> <p>21 Objection to the form.</p> <p>22 A Yes.</p> <p>23 MS. O'DELL:</p> <p>24 Q And -- and in terms of Dr. Longo's</p>	<p>1 Q And did you have the opportunity to</p> <p>2 consider his report prior to finalizing your</p> <p>3 report?</p> <p>4 A I did.</p> <p>5 Q I have nothing further. Thank you.</p> <p>6 EXAMINATION</p> <p>7 BY MS. BROWN:</p> <p>8 Q Dr. Levy, would you take Exhibit 16</p> <p>9 out, please, the FDA's response to the citizens</p> <p>10 petition?</p> <p>11 A I have it.</p> <p>12 Q Counsel asked you some questions that</p> <p>13 involved questions that I asked you. Remember</p> <p>14 she asked you the lawyer for J & J didn't point</p> <p>15 out the articles that were reviewed from 1980 to</p> <p>16 2008 on page 4? Do you recall those questions</p> <p>17 from plaintiffs' counsel?</p> <p>18 A Yes.</p> <p>19 Q Would you look at the last page of the</p> <p>20 letter, page 6 of 7? I'd like to direct your</p> <p>21 attention to the second sentence on this page</p> <p>22 that begins "In consideration of your request."</p> <p>23 Do you see that?</p> <p>24 A I do.</p>
Page 371	Page 373
<p>1 report, his findings of 37 of 56 historical talc</p> <p>2 samples being positive for asbestos and 41 of the</p> <p>3 42 samples tested containing fibrous talc,</p> <p>4 was -- was that information you had prior to</p> <p>5 reaching your opinions and finalizing your</p> <p>6 report?</p> <p>7 MS. BROWN:</p> <p>8 Objection to the form.</p> <p>9 A Yes.</p> <p>10 MS. O'DELL:</p> <p>11 Q And in relation to Dr. Crowley's report</p> <p>12 regarding the fragrance chemicals, do you defer</p> <p>13 to Dr. Crowley regarding his analysis of the</p> <p>14 fragrance chemicals?</p> <p>15 A Yes.</p> <p>16 Q And did you rely on the opinions he</p> <p>17 reached in relation to the fragrance chemicals in</p> <p>18 reaching your opinions in this case?</p> <p>19 A Yes. My -- my review of that just, in</p> <p>20 addition to deferring it, was -- just made the</p> <p>21 general -- or made the statement that I was in</p> <p>22 general agreement with his opinions in those</p> <p>23 matters, seeing as that's not a -- not an area of</p> <p>24 expertise of mine.</p>	<p>1 Q And it states, "In consideration of</p> <p>2 your request, we conducted an expanded literature</p> <p>3 search dating from the filing of the petition in</p> <p>4 2008 through January 2014. The results of this</p> <p>5 search failed to identify any new compelling</p> <p>6 literature data or new scientific data."</p> <p>7 Do you see that?</p> <p>8 A I see that.</p> <p>9 Q And putting together, then, the</p> <p>10 information from page 4 and page 6, you see that</p> <p>11 the FDA considered literature from 1980 to 2014.</p> <p>12 Is that correct?</p> <p>13 MS. O'DELL:</p> <p>14 Object to the form.</p> <p>15 A Yes, that is correct.</p> <p>16 MS. BROWN:</p> <p>17 Q And what the FDA concluded, contrary to</p> <p>18 your opinion here, Doctor, is that a cogent</p> <p>19 biological mechanism by which talc might lead to</p> <p>20 ovarian cancer is lacking; correct?</p> <p>21 MS. O'DELL:</p> <p>22 Object to the form.</p> <p>23 A That's in this --</p> <p>24 MS. BROWN:</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 374</p> <p>1 Q Directing your attention to page 4, 2 number 4, the conclusion regarding a cogent 3 biological mechanism lacking. Do you see that? 4 MS. O'DELL: 5 Object to the form. 6 A Yes. I see where they -- they made the 7 statement that cogent biological mechanism by 8 which talc might lead to ovarian cancer is 9 lacking and that exposure to talc does not 10 account for all cases of ovarian cancer. 11 MS. BROWN: 12 Q Next, Doctor, do you rely on the 13 findings of the Hamilton article in forming your 14 opinions in this case? 15 A Similar to as we've discussed, in a 16 portion, yes. 17 Q You, Dr. Levy, cannot point us to a 18 single paper showing an inflammatory response 19 leading to ovarian cancer in humans from talc 20 use. True? 21 A There is -- I do not know of a single 22 paper that -- in a controlled fashion in humans 23 provided talc exposure that then was -- 24 subsequently led to cancer in humans. That's</p>	<p style="text-align: right;">Page 376</p> <p>1 talc was causing in the body. True? 2 MS. O'DELL: 3 Object to the form. 4 A I'm aware of a number of studies that 5 looked at inflammatory response in model systems 6 and cell lines, and additional studies that 7 looked at inflammation in humans I believe were 8 referenced. 9 Certainly the Penninkilampi manuscripts 10 described inflammatory observations and -- as 11 well as the Buz'Zard and Lau were on human cells. 12 Q Dr. Levy, is it your testimony that the 13 Penninkilampi meta-analysis of prior 14 case-controlled studies demonstrated a 15 inflammatory response of -- from perineal use of 16 talc that led to ovarian cancer? 17 MS. O'DELL: 18 Object to the form. 19 A No. That's not my statement. It was 20 that those -- those papers reported an 21 inflammatory observation as part of those 22 studies. 23 MS. BROWN: 24 Q Not in the tissue from talc; right,</p>
<p style="text-align: right;">Page 375</p> <p>1 correct. 2 Q Controlled aside, you're not aware of 3 any observational case report, any kind of study 4 that shows talcum powder use causing an 5 inflammatory response leading to cancer in 6 humans; correct? 7 MS. O'DELL: 8 Object to the form. 9 A I would -- my review and development of 10 the biological plausibly -- plausible mechanism 11 examined literature that led to the conclusions 12 described in the report. I'm not aware of a -- 13 The human-based studies were all case 14 cohort and -- or case-controlled and cohort 15 studies that showed an association with talc 16 exposure and cancer, but I'm not aware of a 17 direct study. 18 MS. BROWN: 19 Q There have been some reports of alleged 20 findings of talc in tissues or in other parts of 21 the body. Are you familiar with those? 22 A Yes. 23 Q And you're not aware of any one of them 24 demonstrating an inflammatory response that the</p>	<p style="text-align: right;">Page 377</p> <p>1 Doctor? 2 MS. O'DELL: 3 Object to the form. 4 A It would be those studies in the meta 5 review were not examining the tissue content for 6 talc. So they're unable to make that 7 determination. 8 MS. BROWN: 9 Q So we must be missing. I'm -- what I'm 10 asking you is for any study at all in the whole 11 world that shows that talcum powder in somebody's 12 body causing an inflammatory response that led to 13 ovarian cancer. Can you name one? 14 MS. O'DELL: 15 Object to the form. 16 A I mean, we've -- we've discussed a 17 number of studies that described the risk and 18 association of talc in ovarian cancer. But the 19 limitation of the -- of your question or the 20 limitation of the studies relative to your 21 question is those particular studies may not have 22 also assessed the inflammatory response or an 23 inflammatory response, given the nature of the 24 studies.</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 378</p> <p>1 MS. BROWN: 2 Q Well, we got one. We got the Heller 3 study that purported to find talc in ovarian 4 tissue; right? 5 MS. O'DELL: 6 Object to the form. Different -- 7 MS. BROWN: 8 Counsel, it's form, please. 9 MS. O'DELL: 10 Object to the form. 11 A Yeah. What was the -- the Heller 12 study, here it is. 13 Yes, I recall our discussion of this 14 paper. 15 MS. BROWN: 16 Q Right. 17 And this study reported that there was 18 no inflammatory response around the talc that 19 they claimed to have found in the ovarian tissue. 20 True? 21 A They make those statements in the 22 paper, but the -- the -- I would have some 23 concern with the histological methods, but I 24 would certainly defer to a pathologist in the</p>	<p style="text-align: right;">Page 380</p> <p>1 MS. O'DELL: 2 Actually, that wasn't your question. 3 But you've clarified it, so -- 4 A The -- so you're excluding -- are you 5 excluding cell lines? 6 MS. BROWN: 7 Q Yeah. Human beings. Do you know of 8 any study like Heller in human beings that 9 purports to find talc in human women ovarian 10 tissue that shows an inflammatory response? 11 MS. O'DELL: 12 Objection to form. 13 A I'm not aware of a study showing that 14 specifically. 15 MS. BROWN: 16 Q Counsel asked you some questions about 17 nickel causing inflammation that leads to ovarian 18 cancer. Do you recall those? 19 MS. O'DELL: 20 Object to the form. 21 A No. I was asked if -- if heavy 22 metal -- or components like nickel have been 23 shown to have a potential inflammatory response. 24 MS. BROWN:</p>
<p style="text-align: right;">Page 379</p> <p>1 sense of being able to determine the both 2 presence of talc and the inflammatory response in 3 that. 4 Q So you have some critiques of the 5 Heller study. Is that fair? 6 MS. O'DELL: 7 Object to the form. 8 A I would say I would need a -- I would 9 need a -- a -- I would need a further evaluation 10 of the methodology for detecting both talc as 11 well as inflammation in the same materials using 12 the methods of the Heller paper. 13 MS. BROWN: 14 Q Are you aware of any other paper that 15 you think is methodologically superior that shows 16 the presence of talc in ovarian tissue exhibiting 17 an inflammatory response? 18 MS. O'DELL: 19 Object to the form. 20 A Well, we've discussed the rat studies. 21 MS. BROWN: 22 Q Human tissue. That's my question. 23 A Human -- 24 Q Human tissue.</p>	<p style="text-align: right;">Page 381</p> <p>1 Q Uh-huh. Because you're not aware of 2 any published scientific literature that shows 3 heavy metals cause inflamma- -- inflammation that 4 leads to ovarian cancer; right? 5 A I wasn't asked to -- to review for 6 that. I would state that there's a number of 7 studies that show the role of metals -- 8 particularly chromium -- and its -- and its 9 damaging effect on DNA, which I think by -- would 10 certainly have both an inflammatory as well as 11 carcinogenic effect. 12 Q And we're here on an issue of ovarian 13 cancer. And, as it relates to ovarian cancer, 14 you're not aware of any scientific support for 15 the proposition that heavy metals can lead to 16 inflammation that causes ovarian cancer. Fair 17 enough? 18 A Well, I was -- certainly, I was asked 19 to review the literature to develop a -- and 20 develop conclusions of that literature as it 21 related to a -- a potential or possible 22 biological mechanism. 23 In doing that, in part of that review, 24 we certainly made the observation that talc and</p>

Shawn Levy, Ph.D.

<p style="text-align: right;">Page 382</p> <p>1 its components, as we discussed earlier, may</p> <p>2 have -- there's the possibility of having</p> <p>3 additional component effects, such as heavy</p> <p>4 metals and their effects, asbestiforms and their</p> <p>5 effects and the like; therefore, really</p> <p>6 considering the complete components of the</p> <p>7 product overall.</p> <p>8 Q And, as it relates to the testimony you</p> <p>9 just gave, you're talking about just a</p> <p>10 theoretical possibility; right?</p> <p>11 MS. O'DELL:</p> <p>12 Objection to form.</p> <p>13 A Sure. And, then, from that review</p> <p>14 developing a -- a conclusion of a biologically</p> <p>15 plausible mechanism.</p> <p>16 MS. BROWN:</p> <p>17 Q Has that conclusion been published in</p> <p>18 the peer-reviewed literature, Doctor?</p> <p>19 A No, it has not.</p> <p>20 Q And, in fact, as you -- all of the</p> <p>21 opinions that you gave here today, those opinions</p> <p>22 have not been published in the peer review</p> <p>23 literature. True?</p> <p>24 MS. O'DELL:</p>	<p style="text-align: right;">Page 384</p> <p>1 mineralogy of talc.</p> <p>2 Q And whether what Dr. Longo is finding</p> <p>3 in the samples that he tested is the asbestiform</p> <p>4 or nonasbestiform variety of the minerals, you</p> <p>5 would defer to others? Is that fair?</p> <p>6 A I'd certainly defer to Dr. Longo.</p> <p>7 Q And have you looked at any other</p> <p>8 testing of the samples that Dr. Longo has tested?</p> <p>9 MS. O'DELL:</p> <p>10 Object to the form. Vague.</p> <p>11 A Within the literature, there's -- there</p> <p>12 was a number of tables describing testing,</p> <p>13 described tests from previous testimony.</p> <p>14 MS. BROWN:</p> <p>15 Q Have you looked at the testing that</p> <p>16 public health authorities like the FDA have done</p> <p>17 on Johnson & Johnson's baby powder?</p> <p>18 A I believe some of that was provided,</p> <p>19 yes.</p> <p>20 Q Are you relying on any finding of</p> <p>21 asbestos from Dr. Longo in forming your opinions</p> <p>22 here today?</p> <p>23 A The --</p> <p>24 MS. O'DELL:</p>
<p style="text-align: right;">Page 383</p> <p>1 Object to the form.</p> <p>2 A Not at this time.</p> <p>3 Q Counsel asked you some questions about</p> <p>4 Dr. Longo. Do you recall that?</p> <p>5 A Yes.</p> <p>6 Q You've done nothing to validate the</p> <p>7 findings that Dr. Longo writes about in his</p> <p>8 reports. Is that fair?</p> <p>9 A No, I have not done any experiments to</p> <p>10 validate those findings.</p> <p>11 Q Okay. Are you aware that some of the</p> <p>12 samples that Dr. Longo tests and purports to find</p> <p>13 asbestos were purchased off of eBay?</p> <p>14 MS. O'DELL:</p> <p>15 Misstates -- well --</p> <p>16 A My review of the report, I was -- did</p> <p>17 not include the -- I guess the specific history</p> <p>18 of each of the samples.</p> <p>19 MS. BROWN:</p> <p>20 Q Do you understand that asbestos -- that</p> <p>21 minerals like tremolite or anthophyllite, they</p> <p>22 exist in both the asbestiform and nonasbestiform</p> <p>23 way?</p> <p>24 A I would defer to other experts on the</p>	<p style="text-align: right;">Page 385</p> <p>1 Object to the form.</p> <p>2 A The inclusion of the asbestos, again,</p> <p>3 as -- as -- as we've discussed a few times today,</p> <p>4 the conclusion I developed from the report were</p> <p>5 not dependent or independent of any one or</p> <p>6 another component of -- of the talcum powder.</p> <p>7 As we discussed a bit ago, the presence</p> <p>8 of asbestos as a known inflammatory mediator, as</p> <p>9 well as potential carcinogen, I think just helps</p> <p>10 lend additional support to the biological</p> <p>11 plausibility of the mechanism. But I think that</p> <p>12 biological mechanism is not dependent on the</p> <p>13 presence of asbestos.</p> <p>14 MS. BROWN:</p> <p>15 Q Other than plaintiffs' expert,</p> <p>16 Dr. Longo, are you relying on anything else to</p> <p>17 support the potential for asbestos in baby</p> <p>18 powder?</p> <p>19 MS. O'DELL:</p> <p>20 Object to the form.</p> <p>21 A There's -- so I saw reference to</p> <p>22 asbestos content in some of the other literature</p> <p>23 that was reviewed during the time, and, so, there</p> <p>24 were other publications that made mention of the</p>

Shawn Levy, Ph.D.

Page 386	Page 388
<p>1 asbestos content in talc during the overall 2 review. 3 MS. BROWN: 4 Q Sitting here today, are you aware 5 whether or not that was Johnson & Johnson's 6 cosmetic talc? 7 MS. O'DELL: 8 Object to the form. 9 A I would have to look closely. I'm not 10 aware of that specifically. 11 MS. BROWN: 12 Q Counsel asked you some questions about 13 Dr. Crowley and whether or not you were relying 14 on the opinions he reached. Do you remember 15 those questions? 16 A I do. 17 Q What opinions did Dr. Crowley reach on 18 which you rely? 19 A Dr. Crowley performed an analysis of 20 the fragrance components and made assessments of 21 the individual chemical components and their 22 relationship to -- or I should say their -- their 23 inclusion on various lists for their -- their 24 chemical properties or safety. And in most -- in</p>	<p>1 opinion is independent of Dr. Crowley's findings. 2 Is that fair? 3 MS. O'DELL: 4 Objection to form. Vague. 5 A Well, my -- my -- my opinion, again, 6 similar to -- as we've been discussing that, it 7 considers the totality of the information 8 available, including Dr. Crowley's report, but 9 does not rely on any one specific report or 10 otherwise. 11 And, so, the -- again, restating 12 similar to the asbestos, the presence of 13 potential irritants as another component in 14 the -- in the product just provides additional 15 support for that inflammatory mechanism playing a 16 significant role. 17 MS. BROWN: 18 Q If none of the chemicals Dr. Crowley 19 identified were present in baby powder, would you 20 hold the same opinion of biological plausibility? 21 A I would. 22 Q If no asbestos was present in baby 23 powder, would you hold the same opinion on 24 biological plausibility?</p>
Page 387	Page 389
<p>1 the majority of cases, the chemicals were not 2 listed. In a number of cases, there were large 3 numbers of chemicals listed as either irritants 4 and, therefore, able to cause inflammation, or, 5 in a few cases, as potential carcinogens. 6 And, so, it was that review of that 7 information, similar to our discussions around 8 asbestos, that I included or agreed with his 9 opinions regarding that on the last paragraph or 10 close to the last paragraph of the report that 11 stated I was just in agreement that these -- that 12 those chemicals contribute to the inflammatory 13 properties observed. 14 Q Do you know in what quantity the 15 chemicals Dr. Crowley identifies are present, if 16 at all, in Johnson & Johnson's products? 17 A No. I wasn't asked to provide that 18 review. I would defer to Dr. Crowley's report 19 regarding any quantitative analysis of those 20 chemicals. 21 Q And, as it relates to your opinion, 22 Dr. Levy, it makes no difference whether 23 Dr. Crowley's list has ten chemicals in 24 Quantity X or five chemicals in Quantity Y. Your</p>	<p>1 A Yes. 2 MS. BROWN: 3 No further questions. Thank you. 4 MS. O'DELL: 5 I have just one follow-up. 6 Or do you have anything -- 7 MR. FERGUSON: 8 Nothing further. 9 MS. O'DELL: 10 Excuse me. I'm sorry. 11 EXAMINATION 12 BY MS. O'DELL: 13 Q Dr. Crowley, are your opinions in this 14 case contained in your report as well as in the 15 testimony that you've given here today? 16 A You said Dr. Crowley. 17 Q Oh. Excuse me. Sorry. I had 18 Dr. Crowley on my mind. 19 Dr. Levy -- 20 It's getting late in the day. 21 Dr. Levy, are your opinions in this 22 case expressed in your report and your testimony 23 today? 24 A Yes.</p>

Shawn Levy, Ph.D.

<p>Page 390</p> <p>1 Q And do you hold those opinions to a</p> <p>2 reasonable degree of scientific certainty?</p> <p>3 A Yes.</p> <p>4 MS. O'DELL:</p> <p>5 I have nothing further.</p> <p>6 MS. BROWN:</p> <p>7 Thanks for your time, Doctor.</p> <p>8 I think we're off the record.</p> <p>9 VIDEOGRAPHER:</p> <p>10 We're off the record. The time is</p> <p>11 6 p.m.</p> <p>12 (Deposition concluded at 6:00 p.m.)</p> <p>13</p> <p>14</p> <p>15</p> <p>16</p> <p>17</p> <p>18</p> <p>19</p> <p>20</p> <p>21</p> <p>22</p> <p>23</p> <p>24</p>	<p>Page 392</p> <p>1 ERRATA PAGE</p> <p>2</p> <p>3 I, SHAWN LEVY, Ph.D., the witness herein,</p> <p>4 have read the transcript of my testimony, and the</p> <p>5 same is true and correct, to the best of my</p> <p>6 knowledge, with the exceptions of the following</p> <p>7 changes noted below, if any:</p> <p>8 Page/Line Word(s) to be changed/reason Correct Word</p> <p>9 _____</p> <p>10 _____</p> <p>11 _____</p> <p>12 _____</p> <p>13 _____</p> <p>14 _____</p> <p>15 _____</p> <p>16 _____</p> <p>17 _____</p> <p>18 _____</p> <p>19 _____</p> <p>20 _____</p> <p>21 _____</p> <p>22 _____</p> <p>23 SHAWN LEVY, Ph.D.</p> <p>24</p>
<p>Page 391</p> <p>1 CERTIFICATE</p> <p>2</p> <p>3 I do hereby certify that the above and</p> <p>4 foregoing transcript of proceedings in the matter</p> <p>5 aforementioned was taken down by me in machine</p> <p>6 shorthand, and the questions and answers thereto</p> <p>7 were reduced to writing under my personal</p> <p>8 supervision, and that the foregoing represents a</p> <p>9 true and correct transcript of the proceedings</p> <p>10 given by said witness upon said hearing.</p> <p>11 I further certify that I am neither of</p> <p>12 counsel nor of kin to the parties to the action,</p> <p>13 nor am I in anywise interested in the result of</p> <p>14 said cause.</p> <p>15</p> <p>16</p> <p>17</p> <p>18</p> <p>19 LOIS ANNE ROBINSON, RPR, RMR</p> <p>20 REGISTERED DIPLOMATE REPORTER</p> <p>21 CERTIFIED REALTIME REPORTER</p> <p>22</p> <p>23</p> <p>24</p>	<p>Page 393</p> <p>1 DECLARATION OF WITNESS</p> <p>2</p> <p>3 I, the undersigned, declare under penalty</p> <p>4 of perjury that I have read the foregoing</p> <p>5 transcript, and I have made any corrections,</p> <p>6 additions, or deletions that I was desirous of</p> <p>7 making; that the foregoing is a true and correct</p> <p>8 transcript of my testimony contained herein.</p> <p>9 EXECUTED this _____ day of _____,</p> <p>10 2019, at _____,</p> <p>11 (City) (State)</p> <p>12</p> <p>13</p> <p>14</p> <p>15</p> <p>16 _____</p> <p>17 SHAWN LEVY, Ph.D.</p> <p>18</p> <p>19</p> <p>20</p> <p>21</p> <p>22</p> <p>23</p> <p>24</p>

A	accepted 175:13 263:12,18 286:24 287:19 304:11 access 46:9 86:16 88:3 121:22,23 accommodate 10:24 account 374:10 accumulate 335:1 accumulation 192:5 253:24 254:3 335:12 Accumulations 191:23 accuracy 323:23 341:18 accurate 20:23 47:22 74:10 90:19 98:8 accurately 21:13 22:6 30:7 39:20 46:13,22 47:5 86:18 110:19 132:16 145:3 149:12 154:5 173:23 279:6 achieve 311:10 acquired 51:13 51:13,19 53:9 313:8 act 77:24 78:11 action 5:24 142:7 232:14 268:5 391:12 active 106:19 172:3 235:11 activities 98:23 101:10,11,14 109:20 121:6 258:13 294:11 308:14 366:20 activity 31:12 101:6 188:10	197:10 315:16 319:14 acts 349:16 actual 162:22 212:20 acute 92:4 106:13 115:2 115:14 118:10 171:12,18 172:11 239:20 240:15 241:2 241:17 242:1,7 242:20 243:12 244:3,19 245:9 245:19 246:6 246:15,21 247:4,17,22 248:6,15 ad 101:19 add 126:8 243:8 addition 33:20 101:7 182:23 202:13 203:3,5 358:19 365:10 371:20 additional 18:17 18:22 33:14 34:2 35:20 39:15 43:14 47:23 55:7,14 55:20 82:20 182:21 203:8 204:3 207:24 269:18 335:1 335:12 376:6 382:3 385:10 388:14 additions 393:6 additive 369:6 addressed 220:10 adenoma 192:8 192:8 adjunct 103:11 310:21 311:19 312:14 administration	109:14 adult 99:2,4 adults 323:12 aerobic 318:9 aerosol 200:11 aerosol-based 191:9 affect 165:9 216:22 343:19 affirmatively 10:15 11:17 156:23 302:19 afield 318:24 324:16 Afindeis@nap... 2:12 aforementioned 391:5 afternoon 307:4 age 319:2,2,7,7 319:12,21 322:3 age-related 319:23 agent 315:1 322:16 agents 320:10 aging 318:9,13 ago 9:3 14:19,19 97:14 113:4 129:15 148:23 208:12 367:16 385:7 agree 25:18 32:20 52:7 65:21 66:7,12 66:17,18,21 67:7,17 85:18 105:10,15,15 115:20,23 168:24 169:12 185:16,24 205:2 215:1 221:22 223:15 287:1 305:24 313:20 317:4 318:7 321:5	329:8 349:8,14 355:8 agreed 387:8 agreement 206:11 310:3 371:22 387:11 ahead 53:20 301:15 al 25:3 365:6 al- 206:22 Alabama 1:17 1:21 2:4 7:8 91:6 102:21 103:8,10,13 104:19 108:6 Alabama's 103:16 104:5 104:11 ALASTAIR 2:11 alerting 44:7 ALEXIS 2:18 Alexis.kellert... 2:19 algorithm 284:17 alike 58:13 alleged 375:19 alleles 345:23 ALLEN 2:3 allergies 307:10 Alli 8:2 76:19 ALLISON 2:15 Allison.brown... 2:16 allow 62:21 63:4 115:9 122:2 136:18 240:5 256:4 291:22 334:24 allowed 101:13 292:6 allows 363:11 alterations 330:8 altered 213:23 214:5 216:20
----------	--	--	--	--

alveolar 192:7	362:3	103:20 104:14	60:22 62:1	98:19 323:11
AMERICA 3:2	animals 25:20	128:24 136:13	73:19 83:17	324:6,6 337:3
amount 79:5	27:9 36:2,12	137:5 140:10	84:11 122:19	337:20
96:19 100:23	37:9,10 184:14	146:18 153:10	134:23 154:14	April 359:7
133:2,17 134:4	185:19 186:4	155:21 157:6	160:4 161:22	area 97:22
146:4 147:12	186:16,20	157:12 178:8	169:7,10 172:9	101:15 106:19
151:15 153:5	188:2 207:6	185:23 189:2	197:8 224:10	198:18 199:13
162:21 163:3	210:3	206:21 214:22	305:7 327:11	318:19 371:23
163:24 166:8	Anne 1:19 3:21	231:16 235:21	329:2 357:20	areas 31:12
248:3,23	391:18	242:17 243:23	APPEL 3:14	107:23 172:3
299:18 354:3	annual 319:1,7	256:4 262:6	applicable 190:5	283:6
354:12	319:23	294:6	application	argued 238:19
ample 113:23	answer 9:13	answering 70:7	229:5	238:21
129:21 175:9	20:22,23 21:13	71:16 81:15	applications	argument
ampli- 243:8	22:5 25:24	147:11 201:20	227:12 234:5	235:19
amplification	28:2 29:5,9	314:13	applied 180:14	ARPS 2:20
234:23 235:14	30:4,7 38:15	answers 10:13	198:22 222:16	arrive 117:19,22
235:19 243:5	39:19 41:18	10:19 11:12	234:4 360:19	118:17 150:14
amplified 323:2	42:2 46:22	115:16 391:6	apply 88:24	arrived 296:16
amplifying	47:5,22 50:7	anthophyllite	106:18 139:4	art 8:22 9:21
234:20	50:15 55:3	383:21	214:7 226:2	32:10 107:7
analogous 98:5	67:15 69:10	anti-inflamma...	appointed 311:3	329:22 334:3
98:14	86:18 100:4,5	268:21 269:16	appointment	article 5:7,11,15
analogy 320:18	104:17 105:8	antioxidants	311:6	6:11,17,19
analysis 105:6	110:18 128:18	258:13	appointments	30:18 31:19,23
128:17 151:4	130:16 132:15	antitumor	103:11,12	32:1,6,18 34:6
216:22 231:23	139:9 144:16	349:17	appreciate 79:2	37:24 75:6,13
256:9 286:16	145:3 149:11	antitumorigenic	324:13	75:17,22 76:5
323:18 348:11	149:12,23	349:10	appreciated	76:15 80:23
348:13 371:13	154:4,4,8	anybody 74:23	105:24	90:14 123:16
386:19 387:19	155:7,13,23	356:14	appreciates	124:1,4,6,10
analyzed 23:22	159:23 163:6	anywise 391:13	348:7	124:11 125:2,5
and/or 46:6	165:24 170:2,9	apologies 41:10	approached	125:24 141:18
77:16 78:6	170:22 184:19	apologize 307:9	16:20,23	143:23 185:7
94:4 160:20	197:16 217:22	307:11 328:6	approaching	186:23 187:4,9
178:13,19	228:2 231:18	334:17	26:19	187:11,16
260:18 338:21	251:11 291:15	apparent 269:6	appropriate	188:1,16,18
animal 24:18	291:24 293:7,8	269:6	34:23 35:1	207:4 218:14
36:11,19 37:2	293:15 294:21	Apparently	283:9 285:23	218:18,21,24
37:7 120:1	319:22 325:1	56:13	330:1 331:9	219:6,9,11
128:9 183:19	339:17 341:20	appear 31:1	341:6	235:17 237:18
184:5,21 185:2	347:5 353:1	64:18 66:23	appropriately	250:1,13
186:9,10	answered 54:23	172:6 254:16	299:22 334:8	252:23 253:1
207:13,20,24	67:4,11,12	319:17 362:24	appropriateness	267:14 271:9
210:1 303:13	68:16 69:7,8	appeared 85:7	297:23	326:21 327:7
304:5 350:4,10	79:1 80:14	120:11	approximately	327:11 333:4
350:13,16,20	81:2,22,24	appears 18:23	1:18 43:19	339:3,9 374:13

articles 35:8 48:8 75:6 81:7 87:4 144:9 187:8 203:6 263:15 305:13 372:15	128:23 130:17 132:11,20 133:15 134:9 136:12 137:5 137:17 140:10 143:9,10 145:21 146:9 146:18 148:13 150:23 151:20 152:6 153:10 153:11 155:21 155:23 157:18 159:2,6 161:13 161:20 165:6 167:1 178:8 185:22 187:6 189:2 198:4,8 206:21 214:21 228:17 231:16 231:19 234:4 242:16 243:22 254:7 261:8 262:5,7 266:7 285:18 290:6,8 292:10 294:5 297:20 342:6 352:15 354:3 354:16 357:11 364:20 365:18 367:14 372:12 372:13,14 380:16,21 381:5,18 383:3 386:12 387:17	262:20,21,22 263:5 295:4 assault 77:16 assay 212:13 259:3 309:24 assays 319:3 assess 131:19 167:1 213:10 assessed 285:6 377:22 assessing 119:7 assessment 4:21 19:14 179:15 180:13 256:10 285:8 assessments 224:19 285:10 386:20 assist 11:12,18 13:19 assistant 310:16 310:19,19 311:2,6 assists 30:17 asso- 233:13 associate 310:20 311:16 associated 27:10 183:16 205:5 264:20 265:17 266:15 267:16 268:22 273:3,4 274:24 276:8 278:6 290:5 346:17 347:19 association 5:4 5:18 23:11 30:20 83:10 84:13 219:12 220:23 221:2 223:16 224:20 224:23 226:12 227:7,8 228:8 233:19 273:6 276:19 279:24 282:20 313:21 313:22 319:12	347:24 368:14 375:15 377:18 associations 96:15 233:14 330:23 368:20 assume 216:19 342:6 assumed 199:16 assuming 276:17 321:24 368:6,7 assumption 126:12,19 127:13 assumptions 292:9 attempt 133:17 147:1 160:20 231:20 293:3 attempted 105:1 130:6 146:15 156:5 159:22 161:16 177:11 attempting 131:12 attention 33:12 72:12,19 78:3 144:9 198:21 237:24 353:19 372:21 374:1 attorney 96:18 attorney-client 291:4 attorneys 11:24 14:2 93:10 95:5 111:22 123:8,15 attributed 262:20 263:4 atypia 31:11 187:19 188:10 August 41:7 42:23 Austin 3:4 author 37:15,20 84:15,17 184:4 209:22 252:20	303:24 authored 142:23 302:24 authoritative 58:24,24 109:24 authorities 107:12 111:7 384:16 authority 109:11,13 110:1 authors 30:20 32:11,22 33:1 34:13 35:14 38:17 48:13 213:15 219:13 237:21 238:2 265:13 266:17 267:9 269:24 277:3 278:13 280:22 363:13 363:14 autonomy 290:13 available 22:5 26:24 40:1 44:4 46:7 48:18 86:6,10 86:13,14 95:6 119:9,15,21 120:10,14 121:14 131:19 133:22 136:16 139:2 145:10 148:3 149:7 199:4 204:12 220:1,5 223:10 223:22 224:16 225:8 228:6 229:17 245:24 256:13 268:1 289:22 293:18 310:1 362:3 366:1 388:8 Avenue 2:17 3:4 averaging
--	--	---	--	--

234:10	166:21,24	367:7	46:5 57:9	111:18 115:7
aware 9:6 21:4	169:18 174:3	baseline 214:18	58:22 71:13	117:7 121:3
37:7,13 49:10	197:13 200:7	basic 52:17 56:8	85:3 86:11	127:24 130:24
96:13 101:23	237:13,16	65:6 68:16	101:3 110:6	140:14 142:6
102:6 103:15	242:4 243:4	108:20 109:1,1	114:7 116:17	143:18 156:9
103:21 107:1	270:5 302:7,10	178:24 284:13	117:24 120:5	165:5 167:12
108:5 128:13	313:13 319:20	329:19 334:8	123:1,4 128:3	168:1 176:16
136:19 162:17	328:23 330:14	basics 62:17	147:12 158:3	177:13 201:9
165:14 181:11	356:23	65:8	161:24 167:22	201:17 202:1
183:19 184:1	background	basing 199:2	178:4,9 194:9	203:14 204:18
207:5,24 208:3	320:23 366:18	basis 5:17 27:5	199:15 201:14	205:19 206:18
213:7 222:10	balance 275:15	59:4 83:9	211:19,20	225:11 230:3
222:13,18,23	276:1	84:13 86:1	220:18,24	232:21 233:2
234:3 251:8	ballpark 18:21	139:20 140:5	230:1 249:8	234:8 235:16
288:14 292:6	bar 223:24	169:20 179:9	253:2 328:9	247:23 248:14
292:21 296:15	224:13	216:11 287:4	336:20 376:7	261:16 270:11
297:10 304:14	barely 281:6,11	343:14 366:6	384:18	278:1 285:11
304:19 314:5	bark 213:11,18	BEASLEY 2:3	believed 305:21	288:15,23
318:6 353:15	BARRIE 2:8	becoming	Belot 182:22	290:7 313:14
354:24 355:4	BASC 73:5,14	363:17 364:5	benign 249:17	318:14 319:2
375:2,12,16,23	base 110:9	began 293:17	340:3 369:9	373:19 374:3,7
376:4 379:14	based 26:18	352:18	Benjamin 2:21	375:10 381:22
380:13 381:1	53:8 101:17	beginning 9:14	Benjamin.hal...	385:10,12
381:14 383:11	119:7 121:5	24:13 33:16	2:22	388:20,24
386:4,10	126:12,18	105:24 122:18	best 22:15 237:3	biologically
	127:12 128:6	191:22 205:1	268:11 293:15	95:11,11
B	130:6 135:20	238:18 266:12	309:1 312:12	101:16 112:8
B 3:14 122:9	136:14 138:19	362:23 368:19	392:4	113:3 114:3
212:8 340:9	140:13,16	369:2	better 12:8	126:3 129:17
baby 126:12,19	148:2 151:24	begins 31:5	319:16	135:17 136:3,7
126:24 127:13	154:20 162:10	33:14,20 51:3	beyond 109:1	137:9 140:20
128:3,20 130:9	164:20 165:3,4	51:8 53:22	235:2 239:24	143:11 145:22
130:20 134:5	178:23 191:9	72:14,20 76:10	bigger 154:19	146:2 150:20
134:18 135:1,8	199:4 202:8	77:4,10 112:11	binder 13:23	155:24 157:10
137:3 161:8	203:12 204:2	187:17 196:5	14:4	163:2 164:17
178:2 384:17	221:4 226:9	203:3 238:1	bio- 168:22	167:3,8,11,23
385:17 388:19	229:16 235:4	265:4 269:15	Biochemistry	168:4,22
388:22	245:6 254:17	272:20 278:14	25:10 88:17	184:10 188:13
BAC- 73:5	255:12,12	280:14 281:4	bioflavonoids	205:12 219:2
back 16:14	256:17 259:3	305:22,22	213:17	220:3,20 222:4
19:24 38:3	267:24 285:10	372:22	biolo 218:22	225:5,6 226:15
53:19 55:11	291:11 294:12	behavior 199:3	biologic 95:1	227:14,18
56:23 62:19	294:23 312:2	199:3	biological 5:23	228:11,21
63:1 67:24	319:3,7,13,13	beings 380:7,8	37:3 52:17,20	229:17 230:13
82:15 83:20	328:16 333:5	believe 17:13	91:24 93:11,19	231:24 235:11
92:1 98:7	334:3 337:6,6	20:13 24:4	94:1,18,21,24	257:15 260:20
106:3 132:4	352:5 364:24	39:3 44:20	95:9 96:3,8,21	267:3 268:1

275:8 282:11	233:20	British 25:4	94:15 95:13	202:22 203:19
288:1 289:23	bottom 195:5	broad 98:10,23	97:17 100:6	206:16 207:3
290:4,8,14,18	196:3 201:8	120:3 210:7	104:2,9,24	207:15 208:9
292:3 293:3	219:19 327:12	Broadhollow	105:17 108:4	208:19,23
294:1,13,16	331:24 332:8	2:10	110:21 113:14	209:12,19
295:8 296:16	343:6	broken 79:14	114:6 116:16	210:22 213:4
325:23 366:6	BRCA 338:9	bronchial 192:8	117:17 118:6	213:19 214:23
382:14	339:20 344:8	brought 14:20	119:5 120:13	215:14,24
biology 62:17	BRCA1 72:14	15:7,13 39:17	126:17 127:11	216:8 217:8,16
65:14 79:11	72:20,24 73:9	Brown 2:15 4:4	128:2 129:11	218:4,11
81:6 169:6,21	321:2,12,13,17	4:7 7:23 8:2	130:18 131:10	220:17 221:8
175:13 189:20	334:21 335:11	12:10 13:8	131:21 132:6	221:17,21
215:3 217:6	335:16,24	14:13 15:9,22	133:16 134:2	222:17 223:4
263:13 323:5	336:7,8,13	16:6 18:11	134:13 135:2	225:9 226:3
348:7	337:3,13	19:9 20:18	136:5,23	227:16 228:9
bioRxiv 121:11	338:21 339:7,8	21:1,16 22:7	137:20 139:11	229:1,10,21
Birmingham	339:18 340:9	23:5 26:2	141:1,9 142:4	230:21 231:9
91:6 103:10,13	340:19 341:21	27:15,23 28:4	143:20 144:4	231:22 232:5
bit 168:12	342:17 345:7	28:8,14,18,22	145:4 146:3,12	232:15 236:14
186:14 238:14	346:4,13	29:8,14,21	147:3,18	236:22 237:15
307:23 310:12	BRCA1-assoc...	30:15 31:24	148:10,24	238:20 239:13
316:2 319:21	73:3,12	32:16 33:9	149:13 150:9	240:14,23
322:21 327:24	BRCA2 334:21	34:19 36:9,18	151:13,22	241:9,23
336:16 346:9	335:11,17,24	37:6,19 40:21	152:13,22	244:17 245:16
368:13 385:7	336:7,8,13	41:16 42:1,7	153:13 154:23	247:5,15 249:7
blacked 18:2	337:4,14	42:11,18 44:1	155:8,16 156:4	249:23 250:7
Blount 123:17	338:21 339:8	44:16,21 46:14	156:19 157:5	250:11 251:2
124:6,10	340:9,19	46:23 48:12,23	158:2,8,18,23	251:12,18
board 299:14	342:17 346:4	49:19,24 52:23	159:14 160:8	252:10 254:1
bodies 248:14	break 10:23	53:11 54:18	160:18 161:15	254:19 255:5
266:14 356:1	37:24 50:16	57:21 58:15	163:19 164:7	255:16,22
body 169:13	79:18,24 82:9	59:9 61:6,14	164:22 166:23	257:14 258:1
170:14 173:3	88:2 158:6,12	62:18 63:11,19	169:22 170:11	258:17 259:15
210:8 231:1	158:14,21	65:18 66:1,11	171:11 173:9	261:9,20
239:10 249:9	166:14 236:16	66:20 67:6,14	174:16 175:3	262:12 263:1
250:16,19,24	237:1,1,6,17	68:2,20 69:3,9	176:11 178:3	264:8 265:12
251:15 253:5	292:24 301:14	70:15 71:10	178:14 179:8	266:6 267:6
254:3,23 255:7	301:16 356:16	72:5,17 74:19	181:7 182:15	268:8 270:24
257:2,19,24	Breakthrough	75:20 76:21	184:7 185:15	271:18 272:16
258:15 267:20	89:4,14	77:3,7,13	186:1 188:11	275:22 276:24
270:17 320:20	breast 89:4,9,14	78:20 79:22	189:6 190:6,18	279:17 280:11
353:24 356:8	89:18 90:23	80:9,17 81:8	191:5,12,24	280:18 281:1
362:8,14,16	91:9 321:3	82:8,17 83:1	192:16 193:2	281:17 282:14
375:21 376:1	336:10 346:6	87:24 88:10	194:6,21 195:2	284:19 286:2
377:12	bridged 92:6	89:21 90:12	196:2 198:3	286:22 287:8
book 86:24	bring 14:23	91:19 92:8	199:14 200:22	288:12 289:7
bookends	220:3	93:2,15 94:5	201:12 202:10	289:14 290:2

290:23 291:7	214:9 216:9,20	94:4,11,19,21	178:6,12,19	266:15 267:17
291:19 292:14	216:22 217:18	95:2,7,12,23	179:2,11,19	268:23 269:8
292:22 293:24	236:5 241:10	96:6,10,16	181:18 182:12	269:10,17,20
294:15 295:14	241:11,14,19	99:4,5,6,7,20	182:20 183:10	270:10 271:5
295:22 296:8	359:14 376:11	100:2,2 101:17	184:12,16,23	271:13,22,23
296:14 297:9		102:8,22 103:8	186:12 187:1	273:1,4,7,12
298:5,18	C	103:14,17	188:3,14,24	273:16,18
299:24 302:9	C 2:1 3:1 391:1	104:5,11,12,20	189:5,9,20	274:5 275:1,13
302:22 303:10	391:1	104:21 105:2,6	190:3 191:15	275:16 276:3
304:1,21	CA 179:20	105:11,18,22	193:10 201:11	276:10,20,23
305:10 306:11	183:13	106:1,17,18,20	201:19 202:3	277:6,19 278:6
306:20 307:16	calculate 131:12	106:22,23,23	203:16 204:15	278:12,17
350:12 356:13	133:10	107:8,13,17,22	204:19 206:19	279:8,16,24
357:23 358:9	calculated 134:4	107:23,24	207:2,7,14,19	282:1,17,22
359:4,24 361:6	calculations	108:6,7,10,12	208:16 209:3	283:1 284:4,13
363:2 364:16	134:10,12	108:17,18,21	211:12 213:6	285:3,13 289:6
365:15 367:1	calendar 101:5	108:24 109:2,7	217:7,7,11	289:24 290:5
368:3 369:18	call 17:11 217:3	110:5,10,14,24	218:15 219:3,8	293:19,19
370:9,20 371:7	217:19 329:18	111:9,15,17	219:13 220:21	305:21,23
372:7 373:16	called 307:24	112:2,9,22	220:23 221:11	310:10 313:21
373:24 374:11	Canada 4:20,23	113:6,13,17,22	221:24 222:20	314:3,7,14,20
375:18 376:23	19:13 21:21	114:9,17 115:5	223:11,17	320:5,7,17,22
377:8 378:1,7	cancer 4:24 5:5	115:19,19,22	224:3,21,24	321:3,8,15,24
378:15 379:13	5:11,19 6:9,15	116:8,18,21,21	226:19 227:9	322:7,15,17,20
379:21 380:6	20:12 21:7,22	116:24 117:2,5	227:15 228:8	322:24 323:4,8
380:15,24	23:12 25:8	117:10,16,18	228:12 229:7	323:11,13,13
382:16 383:19	26:5,8,15,17	117:21 118:2	230:24 233:9	323:15,23
384:14 385:14	32:12,20 35:12	118:12,13,14	233:22 237:23	324:3,4,12,15
386:3,11	36:2,12,22	118:14,17	238:4,10 239:5	324:16,21
388:17 389:2	51:14,20 52:17	119:19 120:7	239:16 240:5	325:6 326:3,13
390:6	52:21 53:10,15	120:24 121:5	242:11,14	327:1,13 328:2
Brown's 313:4	53:16 54:3,4	128:5 135:20	243:16,17,19	328:14 329:1,7
bucket 106:22	55:8,15,21	136:8 156:2	245:15 246:8	330:1 331:6
bullet 63:21	56:9,18 57:2	159:2,18	246:17,18	333:5 334:7
bunch 277:1	57:13 59:13	161:10 162:21	252:2 253:8	335:7 336:2,10
burden 323:6	60:11,15,22	164:9 165:23	254:4 255:15	336:11,14,20
369:9	61:13 62:17	166:3 167:4,24	255:21 256:1	336:23 337:2
BURNS 2:7	65:8,13 74:13	168:4 169:1,3	256:17 257:18	337:11,11,20
bursal 363:7	79:11 81:6	169:5,6,9,15	259:18 260:6	338:1,10,12,22
bush 154:16	83:11 84:15	169:21 170:15	260:10,12,21	339:23 340:2,7
business 17:12	89:4,10,14,18	170:22 171:1,8	260:24 261:5	340:15 341:7
Buz'Zard 6:7	89:24 90:4,8	171:8,10,12,14	261:17,23	341:17 344:2
181:20 182:11	90:14,18,21,23	172:1,6,16	262:1,3,14,20	344:24 345:7
182:16 208:11	91:3,5,7,8,9,13	173:5,13 174:6	262:23 263:4,5	346:5,7,18
208:14 209:1	92:2,4,6,7,11	174:10,18,22	263:11,13,19	347:2,11,20,24
209:14 210:24	92:15,21,23	175:1,6,10,13	264:5,6,19,22	348:12,13
211:1,10,13,17	93:6,14,20	175:16 176:19	265:3,18	349:23 350:6

350:18 360:16 363:19,23 364:4,7 366:2 366:11,13,14 368:20 369:10 369:10 373:20 374:8,10,19,24 375:5,16 376:16 377:13 377:18 380:18 381:4,13,13,16 cancer-causing 56:19 57:3 313:17 314:11 320:10 322:8 cancerous 37:10 62:22 63:4 64:10,23 115:9 335:3 cancers 105:21 118:18 205:8 206:9 217:15 259:22 canner 321:18 CAP-accredited 309:10 capsule 362:20 carcinogen 338:13,23 340:7,15 344:2 344:6,6,15,19 345:1,13,17 385:9 carcinogenesis 239:1 351:2 353:21 carcinogenic 197:10 381:11 carcinogenicity 352:10 carcinogens 344:4,14 387:5 carcinoma 192:8 331:12 331:22 carcinomas 328:20	care 3:11 108:19 109:1 210:6,23 career 90:15 careful 210:11 210:16 carefully 351:20 carrier 342:17 carriers 337:3,4 338:10 carry 321:23 carrying 362:12 case 1:7 8:12,14 8:20 9:1,5,10 13:16 16:21 18:15 19:23 20:1 24:22 33:5 38:6 39:23 44:24 45:5 47:15 57:13 86:8 90:10 96:4 100:15 101:24 102:2 110:3 111:5,24 120:20 121:17 123:9,10 124:2 124:4 125:3 126:1,11,18 127:12 128:8 129:13 130:21 134:14 135:6 137:1 141:15 157:6,12,24 159:3,16 161:7 164:8 167:1,21 172:5 173:3 176:6,7,13 177:24 182:11 183:14 188:12 191:14 192:18 193:8 195:9 196:20 197:19 198:9 199:5,17 200:15 210:4 219:1 220:1 224:16 227:11 246:14,14	252:24 270:13 275:7,18 288:4 288:6 302:24 305:12 352:5 365:21 366:24 370:8,13 371:18 374:14 375:3,13 389:14,22 case-control 120:2 224:16 228:6 case-controlled 375:14 376:14 case-specific 257:5 cases 1:8 66:6 121:24 169:8 179:15 181:24 190:4 299:12 330:7 338:4 374:10 387:1,2 387:5 caught 353:18 causal 118:24 119:4 205:16 205:16 221:11 221:23 222:6 223:16 314:6 336:5 causality 205:10 causation 221:1 221:4 222:15 223:3 224:13 261:8 262:9,11 264:7 causative 260:14,23 265:10 cause 21:7 26:15 51:14,20 53:10 55:8,14,20 62:20 63:3 91:13 94:3,10 94:21 95:2,12 96:4,5 102:8 105:7,10	106:15 107:5 107:22 112:5 112:24 113:1,6 113:12,22 115:3,18,21 117:5,21 118:1 128:4 129:20 136:8 137:23 140:19 146:5 146:14 147:21 148:9 151:12 151:16,24 153:7,16,18 154:21 155:1 155:10,19 156:18 157:7 157:14 159:1,2 159:17,18 161:9,10 165:8 165:13 169:9 171:1,12 172:1 180:1 184:12 186:12 189:24 208:16 219:3,4 219:7,8 220:21 226:18 228:12 229:7 230:23 230:23 231:24 232:8,20 239:15 241:2 242:22 244:4,5 245:9,19 246:7 246:11,13,21 248:3,6 250:24 252:4 253:16 254:3 255:8 259:10 260:16 260:20,24 261:3,22 262:3 262:14 264:2 264:22 271:23 283:17,23 315:11,12 320:22 327:2 336:14 354:21 363:19 366:10 366:11,12	381:3 387:4 391:14 cause-and-eff... 112:16 313:23 caused 36:21 60:16,22 107:13 118:18 140:8 168:16 173:13 178:4 182:18 183:8 183:20 260:10 262:23 263:19 278:9 335:7 364:21 causes 32:20 36:12,21 60:4 60:15 92:11 102:7 105:2,18 105:20 106:16 110:4 111:15 111:17 112:2 112:21 113:17 114:9 115:4 120:24 145:16 147:13 149:2 149:16 150:15 162:20 165:20 168:9 169:1 174:12 175:7 176:17 177:14 179:1,10 182:20 184:11 184:15,16 186:12,24 188:14 189:9,9 207:18,18 209:2 211:11 211:12,14 222:20 227:3 235:3 241:17 245:22 252:1 255:13 261:5 314:11 336:1 355:9 358:21 365:2 367:8 381:16 causing 37:8
---	---	--	---	--

92:14 94:19	cellular 25:9	106:24 109:5	57:22 106:8	children's
96:9 167:24	31:7,11 106:8	110:7 111:10	111:20 114:1	323:20
168:3 171:10	106:9,10	114:18 115:3	312:15 315:15	choices 319:20
171:21 172:11	113:24 114:1	116:2,15	315:16 318:18	chromatid
173:13 183:21	115:1 164:19	118:13 122:23	321:18 341:18	355:9
209:8 233:9	169:4 171:20	123:5,15 124:8	changed/reason	chromium
242:8 315:1	171:21,22	129:19 133:21	392:6	235:12 243:6
365:12 375:4	172:12 177:24	135:22 137:18	changes 25:20	353:18,23
376:1 377:12	180:15 187:19	145:9 157:22	26:11 27:10	354:4,13
380:17	188:10 189:20	164:14 165:10	30:1,21,23,24	366:23 367:12
cautious 172:17	229:18 238:21	167:16 176:22	32:3,24 34:14	381:8
264:2	239:21 242:22	180:12,21	36:12 37:10	chronic 6:13
cavity 205:5	242:23 243:8	184:18 189:18	60:16,23 94:21	20:10 21:6
363:12	244:4,5 246:13	191:9 198:20	95:1 114:5	24:2,16 26:4
cell 61:17,19	246:16 248:17	199:11,24	115:5,9 183:21	26:10,14 29:24
62:5,21,22	315:1,1,4,11	200:18 209:10	185:19 186:4	32:2,19 36:1
63:3,4 106:1	315:15,20	209:17 211:7,8	188:3 190:9,24	36:20 37:8
182:9 210:2,4	316:3,4,14	215:6,12 221:2	229:19 314:18	92:3,9,14
210:12 212:7	318:5 344:12	223:12 225:24	314:19 363:14	102:7 106:13
212:12,15,16	344:16 348:4	226:11 228:3	392:5	114:9,13,15,22
215:11 217:3,4	360:22 361:14	229:23 242:18	changing 217:6	115:6,14,21
217:6 218:1,5	362:14	247:19 248:21	channel 363:10	116:6,7,17,19
218:6 241:18	center 91:5,7	259:20 260:6	chapter 86:24	117:4,9,20,24
250:17 270:10	102:22 103:8	266:9 283:6	characteristic	118:8,9,18
316:11 317:5,8	103:14,17	284:23 285:21	346:24	136:6 137:23
317:11,17,18	104:5,11,20	299:13 304:7	characteristics	140:7 151:17
323:7 328:19	108:6,7,9,10	305:24 312:17	209:24	151:24 153:7
360:13,16,21	108:12,14,17	326:4 337:18	characterizati...	153:18 155:1
360:23 361:1	108:24 109:7	355:2 363:21	367:11	155:10,19
376:6 380:5	109:14 310:14	366:17,20	characterize	157:8,14
cell's 64:8,20	Central 85:6	367:4 376:9	103:7 104:23	159:18 161:9
316:7	certain 14:23	378:24 381:10	312:17	162:20 164:1,6
cells 60:17,24	15:5 43:24	381:18,24	CHAREST 2:7	164:10 167:23
64:10,22 76:16	53:16 54:4	384:6	check 39:9	168:2 169:8
77:15,17,19	56:19 57:2	certainty 390:2	301:11	172:8,12 178:5
78:5,7,9	109:6 146:4	Certified 1:20	chemical 147:22	180:1 184:11
171:24 182:12	213:10 221:10	391:19	148:8 315:15	186:24 188:14
182:14 209:13	320:6 322:2,8	certify 391:3,11	386:21,24	191:14 207:18
209:21,23	certainly 27:16	cetera 10:1	chemicals	208:15 209:2
212:22 213:22	38:20 43:16	98:10 130:15	235:13 371:12	211:11 219:7
216:21 217:17	47:12 50:18	226:7 230:19	371:14,17	226:18 229:6
269:10 270:5	54:16 57:8	234:18 258:13	387:1,3,12,15	230:23 238:8
270:13 286:1	75:2 79:2,8	challenge 224:6	387:20,23,24	239:4,11,15
316:6 335:1,2	89:10 91:7	challenges 256:7	388:18	240:1,2 242:8
335:12 360:11	92:2,5 96:14	Chan 328:15	Chicago 3:9	243:13,18
360:14 361:4	97:23,24 103:9	change 33:2	childhood	244:5,19
365:9 376:11	103:22 105:4	35:15 37:5,17	323:13,15	245:10,19

246:6,15,22	clarification	308:6,17,22	come 99:22	community
247:4,18,22	132:10 215:2	309:2,6,7,11	283:5 284:14	287:1,20
248:3,18 261:3	clarified 380:3	309:13,17,20	284:14 285:4	company 307:7
261:5 266:13	clarify 34:21	309:24 310:4	366:20	compare 47:3
267:19 268:24	132:20 154:10	close 45:15	comes 70:18	318:2
269:19 270:8	169:4 202:7	301:20 303:11	98:12 299:11	compared 200:1
271:3 275:15	233:5 246:16	327:21 387:10	317:8 325:8	337:10
276:1,9 278:4	268:15 346:10	closely 386:9	344:20	comparing
279:7 281:23	365:22	coalition 193:11	comfortable	86:19 215:22
282:23 284:2	clarity 135:16	cobalt 366:23	337:19	263:16
285:10 313:15	classical 168:13	code 62:3 100:7	coming 117:4	comparison
351:2,7	classification	100:18,20	140:7 282:4	236:5
circumstances	108:11 110:24	101:18	comma 53:15	compelling
252:3	305:4	codirector	54:3 55:7,13	193:20 373:5
citations 331:17	classified 328:16	104:18	61:20 62:6	compensation
332:23	classify 144:10	coexist 308:20	73:10 77:24	292:11
cite 34:6 116:5	clean 42:12	cogent 201:9	78:5,7 278:17	complete 107:16
116:15 123:16	clear 112:7	202:1 203:14	commencing	108:2 110:8
271:9,11 272:5	125:21 128:12	204:18 206:17	1:17	112:4 113:5
327:6 328:15	161:24 166:6	373:18 374:2,7	comment 49:18	123:15 137:13
333:13 338:16	214:14 227:7	cohort 120:2	52:16 68:1	143:13 209:7
341:11 356:5	245:24 274:7	176:4 224:7	131:7 137:17	290:13 353:5,5
358:19 370:18	275:3 278:3	228:5 338:3	253:14 279:15	382:6
cited 12:23 39:5	308:9 315:22	375:14,14	340:4 342:3	completed 40:3
73:24 122:13	328:19	coincidental	347:4	45:4 194:2
123:5 124:7	clearance	333:18	commenting	completely
125:11 203:7	163:14	cold 307:10	85:9	255:7
218:18 253:2	clearly 32:13	collaboration	comments 83:13	completing
265:24 273:10	58:7 65:15	323:19	83:19 84:2,4	22:16
273:14 274:2	324:10	collaborator	210:1 250:14	completion
326:16 339:13	CLIA-licensed	312:19	266:19 335:24	39:21 43:7
339:15 340:5	309:8	colleague	Commerce 2:3	294:23 303:5
358:13 362:2	climate 299:5	301:21 303:12	COMMITTEE	complex 73:3,4
367:17 369:17	Clinic 5:11 59:5	colleagues 87:12	2:2	73:12,13
cites 35:20,24	59:12,20,23	97:8,16 287:15	common 259:21	106:23 107:8
citing 333:10	60:3,14,15	312:20	259:23 260:2	172:17 210:8
334:8	61:16 62:19	collect 320:21	268:19 316:10	265:3 348:2
citizen 202:9,21	63:22 64:5	collection 12:23	316:10 327:3	complexities
citizens 6:5	65:20 68:9	74:8 125:7	commonly	105:23
192:19 195:17	69:5	column 187:17	70:23 309:23	complexity
372:9	Clinic's 58:20	272:18,24	330:24	107:5
City 393:10	58:23 62:11	278:14 368:12	communicating	compliance
claim 338:21	63:14 64:14	com- 235:3	307:20	101:21
353:23	65:3 66:14,24	combined 72:14	communication	complicated
claimed 196:1	67:19	72:21	44:13 363:11	107:3 320:14
378:19	clinical 99:1,13	combines 72:24	communicatio...	complication
claiming 345:10	99:21 227:24	73:9	44:9,15	363:8

complied 100:13	108:6,10,13,23	199:6 203:14	190:5 299:9	consequence
comply 100:17	125:6 127:7	222:19 250:15	323:16 345:6	318:9
component 26:7	145:10 177:22	265:14 355:5	conditions	consid- 173:2
99:6 100:22	301:5 348:19	363:13 373:17	196:15 215:12	consider 58:5,23
118:10,11	comprehensiv...	390:12	216:6 277:2	70:2 97:15
127:8,19	223:13 224:1	concludes	285:11 314:4	109:10 111:6
128:15 129:16	comprising	187:20 206:17	317:15 340:3	138:9 149:1
136:15 150:4	330:19	223:16 275:14	369:9	167:17 188:15
151:1 152:10	computational	281:20	conduct 120:18	192:11 193:6
168:3 169:11	121:7	concluding	194:17 196:8,8	200:14,20
171:8,14	computer 11:16	204:17	223:9 231:23	209:13 224:1
242:18 262:1	30:9	conclusion	conducted	243:1 250:4
262:19 263:3	con- 106:3	27:19 31:18	143:22 204:8	252:23 282:3
315:15 316:24	conceded	35:16 111:13	213:5 373:2	284:20 286:9
317:2 320:9	200:10	117:20,23	conducting	289:8 294:1
321:7 347:1	concentrating	118:17 137:21	231:10	297:7 304:22
363:23 366:8	361:11	137:22 140:7	conference 97:5	305:12 309:2
382:3 385:6	concept 70:6	150:14 160:1	confidence	336:13 343:16
388:13	96:17 225:12	160:16 186:10	279:18 280:6	344:14 361:8
components	289:10,20	201:4 202:1	280:14 281:3	372:2
94:3 126:24	319:1	203:20,22,24	confident	consideration
127:22 135:18	concepts 68:18	204:8,9 205:18	299:20	123:9 286:14
136:17 137:7	178:24	215:13 253:22	confidentiality	305:3 365:12
137:14,16	concern 197:7	259:6 283:4	310:3	372:22 373:1
151:7,12	378:23	304:9 319:23	confirm 99:24	considerations
153:23 154:18	concerned 312:4	374:2 382:14	145:13	146:23
210:18 211:9	concerning	382:17 385:4	confirming 52:5	considered
234:16,21	119:17,18	conclusions	273:5	13:15 24:21
243:6 344:16	concerns 173:18	30:22 32:15,17	conflict 270:21	58:3 96:8
352:20 353:3	206:4,12	36:8 87:11	299:14,16,21	111:10 168:22
355:3 380:22	224:12 228:4	111:6 112:16	confusion	181:12 188:17
382:1,6 386:20	255:3 301:7	126:5 138:15	308:19	192:17 200:18
386:21	312:2	138:19 139:4	Congress 3:4	200:19 202:20
composition	concert 53:10	159:12 173:16	connecting	219:9 249:12
147:8,22 148:8	106:11 115:12	174:5 204:5	178:24 276:15	284:23,24
148:15	244:10	216:16,17	278:1	292:8 295:6
compound	conclu- 138:2	219:11,18	connection	336:1 373:11
37:12 232:20	conclude 32:11	220:16 233:17	13:15 17:15	considering
235:3	35:16,17 153:7	268:12 276:23	25:13 58:21	97:12 227:13
compounding	158:21 200:23	279:3,13,13	93:12 100:14	283:16 365:4
177:4	238:2 261:2,4	283:23 284:15	120:19 192:17	382:6
compounds	265:14 267:9	305:6 366:21	193:7 223:6,14	considers
235:11 367:11	276:1 281:23	375:11 381:20	252:23 275:17	126:23 284:11
comprehensive	282:23 283:17	conclusive 205:9	293:12 304:22	388:7
91:6 102:22	concluded	conclusively	305:11	consistent 15:8
103:17 104:5	110:12 185:18	189:5 215:7	connective	87:2 275:5
104:11,19	194:15 195:20	condition 107:9	362:20	consisting 203:6

consists 112:12	63:13 126:12	295:12	156:2	169:15 170:15
consolidate 106:10	126:19 145:24 150:12 193:4	contr- 206:23	corn 150:1	172:21,22,24
consolidating 75:18	contaminants 137:12 138:21 198:2	contracts 98:13	cornstarch 145:7 148:17	173:1 180:3
constituent 94:3	contaminated 128:21	contradictory 268:16	148:21 149:1	185:20 187:9
95:8 126:24	contaminating 154:1	contradicts 206:23	149:15 150:1,3	188:3 194:9,12
127:22 135:18	contamination 126:7 129:8	contrary 373:17	150:7,12	195:10,18,19
136:15 137:7	130:8 131:4,13	contrast 76:10	Corollary 321:16	201:22,23
137:13,16	131:24 132:19	76:11,16 77:4	correct 10:5	202:3 203:20
151:2,7,11	132:21 133:5	77:10,14 78:4	20:2,3,8 22:22	203:24 211:18
153:23 154:18	133:11 151:6	139:1	31:16,19 34:14	211:24 214:19
234:15,15	278:19	contribute 71:4	35:5,6,21,22	215:8 218:19
352:10,20	content 23:20	242:10 353:12	36:22 39:13,14	218:20 219:18
constituents 114:5 139:3	45:1,12 74:24	387:12	41:11 46:17	219:19 222:20
150:21 243:2	79:6 85:10	contributing 37:4 262:21	52:11 54:8,12	223:17 229:11
constraints 32:8	90:11 102:5	contribution 172:1 353:16	55:2,15,16,18	237:23 239:5
consult 58:20	116:3 121:23	control 214:15	55:22 56:1	240:10,16
69:14	122:1 131:4,24	323:7 348:4	57:7 61:1	241:19 244:20
consulting 101:10,14,20	295:13 339:4	controlled 374:22 375:2	64:15 67:20	244:21 247:8
consumer 138:12,14	377:5 385:22	controlling 92:4	70:21 73:15,16	261:12 262:17
196:13 197:21	386:1	controls 128:9	74:15 78:16	264:17 265:22
198:1 236:3	contents 48:4	192:10 200:13	80:4,11 81:10	265:23 269:22
contact 17:7,13	85:14 102:11	controversial 264:4	81:19 84:9	271:9,14,15
contain 20:10	208:2	conversation 68:1 101:8	85:16 87:15	274:6 280:8
24:1,16 86:3	context 54:17	106:4	88:12,13,14,15	287:15,16,21
127:14 131:9	66:5 70:13	conversations 18:8 291:2	90:1,2,4,16	290:12 293:8
141:14 146:5	81:16 82:1	292:12	92:11 93:23	309:15 310:14
150:6,7 235:6	128:16 225:13	convey 44:13	94:12 95:18	310:15,17
contained 12:5	225:15,22	convincing 194:16 196:6	96:10 97:1,2,5	316:16 320:7,8
13:3 15:5 20:6	349:11	copied 58:11	97:6,9 99:15	321:13 322:10
22:20 39:12	continuation 243:12 265:10	copies 15:21	102:22,23	322:22 326:13
96:23,23 97:4	continue 77:17	copy 13:21,23	107:13,14,17	326:14 327:4,5
97:8,19 124:6	78:8 107:12	16:11 19:12	109:11 113:17	327:8,13,14,16
130:14 136:21	182:6 248:18	30:8,16 44:3	115:8 116:9	328:2,21,22
208:5 211:7	continued 3:1	47:2 48:21	118:2 122:14	329:3,15 330:9
231:11 256:14	5:1 6:1 239:24	83:4 84:23	122:17 132:13	330:10,12,13
282:5,11	240:1 243:10	141:22 191:3	133:8 134:18	330:21 331:13
286:15 334:1	244:6,6 245:23	312:21 368:4,7	135:8 136:10	331:14,18,19
389:14 393:8	264:20 312:14	core 104:18	137:3,6 142:17	332:16,17,19
containing 371:3	continues 136:2		142:19,23,24	332:24 333:11
contains 21:5	200:9 234:14		144:6,7 151:17	333:16 335:4,5
61:18 62:4			152:2 156:22	335:8,9,13
			157:1,4,8	337:5,6,14
			159:19 160:10	338:14,15,17
			160:21 164:20	338:18 340:10
			167:5,6 169:1	343:5,22,23
				344:2,3 346:1
				346:2 347:13

347:14 349:6,7 351:4,5 352:7 352:8,11 354:15 358:11 363:16 373:12 373:15,20 375:1,6 391:9 392:4,6 393:7 corrected 64:10 64:22 corrections 64:8 64:20 393:5 correctly 53:17 54:5 55:9 64:6 76:6 184:22 192:21 212:11 273:8 correlation 222:24 corresponding 84:17 cosmetic 177:7 386:6 COUNCIL 3:11 counsel 2:2 3:7 3:11 7:13 15:12,23 16:8 17:3 18:7,8 19:5 20:17 23:1,1 27:24 28:5,9 38:13 39:16 41:17 42:2 43:3 44:10,15 66:2 82:20 185:11 193:24 275:23 291:3,8,12,14 291:20 292:7 292:12,15 293:2 358:24 359:20 368:4 372:12,17 378:8 380:16 383:3 386:12 391:12 counseling 323:21	counted 47:23 counter 297:1 couple 11:7 224:11 236:1 280:1 369:22 coupled 224:8 course 12:13 32:14 33:2 35:14 74:22 90:14 101:6 123:7 131:6 137:15 163:12 183:4 293:20 298:2 370:5 court 1:1 3:22 7:15 10:1,12 10:20 11:2 182:2 Coussens 5:15 75:7,12 76:2 76:15 77:14 79:7 80:3,22 Coussens' 75:22 76:5 cover 313:3 covered 313:4 create 114:5 created 16:12 creates 174:22 175:5 creating 164:19 creatively 308:16 criteria 108:12 143:18 critical 164:23 172:9 critique 195:17 200:8 critiques 200:14 300:21 379:4 Crowley 40:13 40:14,19,22 43:2,8 45:3 371:13 386:13 386:17,19 387:15 388:18	389:13,16,18 Crowley's 371:11 387:18 387:23 388:1,8 CRR 3:21 current 53:8 101:21 107:7 160:4 169:6 171:7 180:10 260:4 319:14 331:6 333:7 currently 99:3 108:3 309:19 336:21 curve 249:6 cycle 323:7 cyclooxygenase 268:5,18,18 269:10 270:14 270:19 cysts 368:21 cytokines 238:23 316:5 349:5 <hr/> D D 2:8 4:1 5:1 6:1 D.C 3:13 Dallas 2:8 damage 73:1,10 77:15 78:6 106:9,10 113:24 115:1 164:19 171:21 172:12 189:20 239:21 242:23 242:23 243:9 244:4,5 246:13 246:16 248:17 315:3,5,12,17 315:24 316:4 344:8,16 353:20 354:5 damaging 314:23 316:6 381:9 data 85:22 95:22	193:16,19 224:16 240:18 245:12 256:13 260:4 268:1,11 268:16 273:5 279:6 319:5 354:19 373:6,6 date 1:18 7:5 40:22 41:1,1,3 41:6,10,23 42:5,6,10,17 42:20 43:7 85:2 310:2 359:3 dated 16:9,10,17 39:12 83:5 84:6 358:21 359:7 dates 42:14 dating 92:1 373:3 day 142:11 172:16 303:14 389:20 393:9 days 45:22 47:8 dealt 99:11,14 death 212:16 327:2 decades 242:22 December 5:21 18:23 19:19 20:5 23:17,23 43:9 83:5 85:1 85:13 decision 311:7 DECLARATI... 393:1 declare 393:3 declared 201:1 decrease 212:20 214:17 215:2 349:4 359:21 360:22 361:13 361:15 dedicated 308:22,23 defect 344:11	defective 343:12 defects 334:24 DEFENDANT 2:13 3:2 defendants 8:17 Defense 90:24 91:2 271:2 defer 114:20 134:10 148:14 157:22 223:1 233:11 247:2 254:15 262:9 285:21 354:6 370:2 371:12 378:24 383:24 384:5,6 387:18 deferring 127:20 371:20 define 138:6 167:7 201:17 346:22 defining 257:24 355:3 definition 139:3 139:13 150:5 172:10 definitions 317:1 degree 44:12 290:22 291:12 291:16 390:2 deletions 393:6 delighted 326:4 delineate 138:23 177:12 244:2 244:21 247:22 delineated 139:22 235:24 244:9 247:7 delineating 240:6 246:15 delineation 165:15 352:16 delving 314:17 demonstrated 322:14 324:20 354:20 376:14
---	---	--	--	--

demonstrating 375:24	142:2 185:9 190:16,20	designed 129:23 251:11	288:9 323:15 385:4	163:12,16,17 176:23,24
denied 312:10	192:24 208:7	desirous 393:6	developing	177:1 185:3,4
denying 195:16	208:11 218:9	detail 53:7	95:17 96:20	197:23,24
deodorant	218:13 249:21	114:16 137:18	282:10 290:18	198:15 201:21
139:17,23	270:22 271:2	244:24 254:14	322:15 324:21	204:8 212:7
145:16	301:18 302:16	263:17 314:16	336:22,22	213:1 236:2,9
deodorizing	326:18 367:21	339:16	338:12,22	247:7 248:4
139:13 145:6	367:22 390:12	details 157:9	339:23 340:6	259:17 262:16
department	depositions 9:11	184:1	340:15 341:17	265:2 277:2,4
90:24 91:2	derived 294:24	detect 258:24,24	344:24 350:21	294:8 315:8
311:8 312:15	describe 48:11	detected 189:24	382:14	317:1,14
departure	79:10 115:17	detecting 256:7	development	329:23,23
312:18	129:4 135:17	379:10	52:21 60:11	330:19 346:9
dependency	137:17 140:23	detection 184:23	123:7 178:13	360:14,15
215:21 247:21	160:6 263:17	227:9 255:4	178:19 179:2	366:8 378:6
247:21 248:22	289:20 365:20	determin-	238:10 242:11	differentiate
dependent	described 9:10	229:22	269:20 275:16	308:10
134:15 135:7	36:8 37:16	determination	276:3 278:17	differentiates
136:9 149:9	56:11 87:9	97:21 149:10	279:8 282:1,24	247:17
156:10 227:19	114:18 129:18	151:14 153:4	284:3 346:5	differentiating
385:5,12	131:1 140:19	196:19 198:8	347:11 350:5	275:10
depending	147:7 149:16	198:11 199:1	350:18 364:7	difficult 47:21
154:17 316:3	180:11 181:5	228:13 230:4	366:5 375:9	174:7 217:22
317:10 324:7	199:22 204:11	251:3 377:7	devoted 89:24	223:24 224:13
349:4,10	234:23 240:9	determine 59:6	90:3,7	320:1
depends 258:22	242:5,13	132:21 140:3	diabetes 95:24	Diplomate 1:19
348:3	247:11 366:8	155:17 203:8	diagnosis	391:19
deponent 7:12	375:12 376:10	271:20 337:17	106:22	direct 33:11,11
deposed 8:5	377:17 384:13	379:1	dice 320:21	33:18 51:2,7
10:4	describes 48:20	determined	dichotomy	60:2,9 63:21
deposition 1:14	86:24 143:16	193:19 340:20	285:1	63:24 72:6,12
4:13,14,15,17	360:10	342:18	die 322:1 361:5	72:19 75:21
4:19,22 5:2,6,8	describing 56:8	determining	diet 318:17	76:6 99:23
5:10,12,14,16	61:12 62:15	155:18 318:4	difference 57:10	167:20 171:19
5:20,22 6:2,4,6	128:17 129:4	347:1	81:9,12,19	180:13 194:14
6:8,10,12,16	131:14 162:1	develop 27:1	147:14 177:6	203:2 237:24
6:18 7:7 8:11	266:18 360:13	56:18 57:2	317:21 318:5	243:7 250:12
9:24 14:9,10	384:12	95:8 111:18	387:22	272:18 275:23
14:11,22 15:7	description	151:8 164:9	differences	276:19 291:14
15:14 16:4,8	61:13 65:8	175:16 289:19	185:4,5,6	293:8 314:10
19:4,7 21:14	141:14 176:15	290:8,14 292:3	different 24:19	314:23 342:7
22:24 23:2,3	187:15 198:22	293:3,11,16	47:6 58:7,13	350:24 353:15
30:13 33:7	219:22 230:15	322:7 323:13	81:4 82:1 94:6	372:20 375:17
39:18 49:22	design 32:9,14	381:19,20	98:20 108:11	directed 51:18
59:7,11 72:3	194:17 196:7	developed 48:19	115:7 144:10	181:5
75:14 82:19,23	209:18 228:4	151:10 173:5	147:4 154:17	directing 78:3

291:10 374:1	244:23 268:4	238:1 254:15	106:18 154:15	158:24 159:16
direction 170:8	285:22 314:16	265:14 274:15	154:15 162:9	163:24 164:9
directions	320:4 340:1	289:5 291:21	184:21 198:14	166:15,24
119:24	discussed 102:1	297:19 319:11	divide 61:21	168:24 169:24
directly 107:22	106:5 129:15	339:22 359:19	62:7	170:12 171:13
108:18 163:6	153:24 172:16	359:23 360:2	DNA 60:17,24	174:17 184:8
171:1 173:12	179:13 180:7,8	361:22 362:22	61:16 62:2	185:16 186:2
179:6 180:16	185:17 186:14	378:13	64:1,7,8,9,19	186:22 187:5
218:3 228:2	187:12 189:3	discussions 9:22	64:20,21 73:1	190:8,13,19
240:3 314:6,23	205:24 208:4	74:8 92:21	73:10 76:17	191:14 192:1
315:10,11	209:6 216:15	138:21 146:10	77:15 78:5	192:23 194:7
319:22 353:20	226:11 227:4	154:6 197:4	242:24 314:24	195:3,7 197:14
dis- 212:17	227:23 237:22	243:5 266:20	315:2,5,12,17	198:5 199:6
disagree 113:20	279:15 282:8	291:13 315:9	315:24 321:11	200:8,23
116:14 173:16	287:13 295:4	365:23 387:7	323:6 344:7,9	201:20 202:11
197:9 202:6	298:9 303:6	disease 95:23	344:17 353:20	202:23 203:2
203:23 204:1	304:11 336:6	106:1 107:3	355:10 381:9	204:6 205:18
212:17 213:2	342:24 359:6	224:2,7 244:13	DNA-damaging	207:5 214:16
245:5 278:23	363:20 365:6	246:19 256:6,6	243:7	214:24 216:1
283:22	374:15 377:16	260:17 261:1	Docket 1:9 7:11	219:23 221:9
disagreement	379:20 382:1	265:7,10	Doctor 11:6	221:23 229:2
197:3	385:3,7	271:13,21	14:5 15:24	236:17 237:4
disclose 100:21	discusses 24:17	272:8 274:4	16:12 17:22	237:16 241:24
102:13 300:2	discussing 19:13	275:11 276:17	18:13 19:3	246:20 249:24
disclosed 97:7	21:21 23:10	276:21 277:23	20:19 21:11,19	250:12 253:4
101:6 257:5	79:4 175:8	282:21 283:4	22:16,23 24:9	255:6 261:21
297:18 298:1	181:21 189:20	284:6 324:7,9	27:13,17 30:16	266:22 268:10
300:6 309:20	193:5 200:8	327:2 347:2	30:18 31:4	269:14 272:18
310:2,6	201:14,22	348:17	33:4,15 35:23	274:17,19
disclosure	205:14,15,24	diseases 96:1	36:10,20 37:8	276:4 278:14
100:11,19	206:15 220:7	277:17	37:20 38:3	279:19 280:13
101:5,19	226:6 237:18	disorder 265:3	44:18 47:15	281:6,18
102:19 298:12	242:6 245:11	displayed 361:9	50:6,21 57:23	286:23 292:1
298:23 299:3	247:14 258:9	361:10	59:10 60:21	301:23 302:1
discontinued	262:18 263:8	dispute 100:1	61:7 64:11	302:10 304:3
163:15	264:3 267:5,18	254:21	66:21 71:14,24	304:14 305:11
discover 120:17	269:18 277:11	distention 363:7	73:17 74:14	357:8 360:9
131:3,23	277:24 280:12	distinction	76:8 78:4,14	361:18 373:18
132:19	281:20 293:23	115:1 171:5,16	80:1 81:18	374:12 377:1
discoverable	295:2 296:22	191:8 196:22	82:18 84:19	382:18 390:7
292:7,10	296:24 303:5	244:16	85:12 98:3	doctors 89:23
discovered	325:9 350:10	distinguish	104:3 105:19	document 1:7
123:14	353:17 365:5	200:4 317:16	114:7 126:11	4:20,23 19:5
discovery	370:16 388:6	317:20 352:9	132:7 133:6	21:10,13,20,24
291:22	discussion 9:24	DISTRICT 1:1	135:12 137:22	22:14,24 83:21
discuss 44:14	97:16 143:12	1:2	138:5 141:11	142:5,10,12,15
93:13 238:7	166:10 233:3	diversity 91:8	141:22 142:10	142:18,21

193:7 200:7 206:24 document's 193:9 documentation 102:17 119:9 documents 13:15 14:23 39:16 68:15 82:20 352:23 doing 267:4 381:23 dollars 100:24 dose 159:10,12 160:10,15,21 160:24 162:11 212:23 215:22 215:23 275:3 360:5 361:3,12 dose-dependent 360:23 dose-response 249:5,5 doses 180:14 211:18 212:21 215:21,22 360:16 dosing 176:23 double-check 241:4 271:24 Double-sided 60:7 dozen 43:19 Dr 5:21 7:12,24 8:5 15:20 18:8 29:9,22 32:18 40:14,15,19,20 41:12 43:2 44:9 49:4,7,13 49:21 50:10,18 50:24 51:1,18 53:13 54:8,12 55:2,6,18,24 56:15,16 68:22 72:6 76:15 77:9,14 83:6 83:21 84:16	85:18 86:12,23 87:12 88:4,11 93:16 110:3 111:6,14 116:6 117:8 122:21 125:15 130:7 158:14 168:11 170:9 177:21 179:22 180:10 181:8,13,15 183:20 203:23 204:16,16 247:3 260:19 272:15 285:22 286:3,6,10,13 286:15 288:22 291:6 292:12 296:24 297:11 298:1,7,12 299:15 302:12 302:17 307:4 357:3 359:11 362:22 363:18 368:9 369:23 370:12,18,24 371:11,13 372:8 374:17 376:12 383:4,7 383:12 384:2,6 384:8,21 385:16 386:13 386:17,19 387:15,18,22 387:23 388:1,8 388:18 389:13 389:16,18,19 389:21 draft 4:20 5:3 19:14 23:8 45:24 46:4,10 46:24 47:5,6 47:11,16 49:6 drastically 318:18 draw 32:14 215:13 253:22 drawing 174:5	278:3 drive 3:8 12:16 12:20 13:3,9 13:15 40:12 drug 299:9 drugs 268:21 269:16 due 101:2 163:10 185:4,5 266:13 267:19 270:18,18 duly 7:19 dur- 233:1 duration 146:23 152:10 159:13 160:20 161:3 163:3 228:19 230:18 233:2 234:5 275:4 360:5,6 dust 200:13 <hr/> E E 2:1,1 3:1,1 4:1 5:1 6:1 391:1,1 392:1,1 earlier 16:22 22:12,14 35:13 37:15,16 42:21 45:9,11 54:24 74:7 86:16,20 87:3 106:3,3 127:17 140:12 153:24 168:12 172:16 180:8,9 180:19 184:2,3 185:8 186:14 198:21 216:15 224:4 226:11 243:4 258:9 259:9 263:14 263:16 266:20 277:24 282:8 287:5 291:21 301:11 302:12 303:6,14 321:23 335:6	350:9 352:4 357:7,11 361:21 363:20 365:6,23 367:14,15 370:6 382:1 earlier-cited 183:5 earliest 184:4 early 17:7 92:1 176:1,16 190:1 306:4 324:2,4 EASTERN 1:2 easy 125:18 eBay 383:13 EC 328:19 331:13,21 332:14 edit 71:4,11 editable 74:23 educated 198:13 198:18 effect 96:4,5 112:21,24 113:2 114:17 140:19 148:9 163:13 165:14 176:8 213:11 213:14 217:24 232:1,21 233:2 234:13,20,23 235:14 239:20 239:20 243:7 269:15 270:18 314:6 323:2 349:17 369:7 381:9,11 effective 269:8 effects 4:24 21:22 34:2,11 34:13,23 35:4 95:23 129:22 136:20 165:11 176:5 239:19 315:13 317:24 320:10 349:10 355:6 362:4	382:3,4,5 effort 99:4 eight 61:10 eighteen 32:7 either 35:18 39:16 107:21 127:1 131:20 131:20 162:2 163:20 167:12 167:20 171:19 181:24 240:3 244:9 253:7 300:21 387:3 electronic 102:18 electronically 40:2 elevated 273:4 elevation 183:13 elicit 172:24 205:6 264:9 elicits 281:22 ELLIS 3:8 elucidate 221:1 elucidation 249:3 email 5:21 14:18 17:1,13 44:2,6 83:5 Embassy 1:16 EMMEL 2:5 employ 117:3,19 118:16 140:6 188:15 220:8 282:3 employed 141:15 143:2 150:13 204:7 encodes 343:18 endeavor 143:3 231:12 endeavored 177:5 276:7 277:4 endo- 331:12 endogenously 318:3
--	--	--	---	--

endometrial 205:4	108:20 120:6 122:5 144:21	174:11 221:12 221:24 222:15	31:11,14 33:21 110:12 113:11	52:4,6,7,11,15 54:11 80:20,22
endometrioid 328:19 331:12 331:22	145:14 146:24 148:22 152:9 157:21 160:1	226:23 314:15 323:4 347:12 347:17 348:21	113:15,21,23 114:8,15 119:8 119:14,15,17	234:3 334:4 examination 4:2 7:22 95:22
ends 330:11	166:7 173:4	establishing 121:2,13	120:23 129:10 129:12,15,16	307:2 357:1 372:6 389:11
engage 103:5	222:19 223:1,7	140:13	129:21 140:15	examine 96:3
engagements 101:20	223:10 225:1 233:11,14,24	estimate 132:20	142:7 162:13	136:20 157:21
enhance 242:11	254:13,15	et 10:1 25:3	164:20 165:21	160:14 224:1
enter 255:7	256:2,15	98:10 130:15	166:1 167:14	228:1 234:7
enters 249:9 250:19,24	262:10 284:14 288:4	226:7 230:19 234:18 258:13	175:9 177:15 181:16 186:2	365:8,24 examined 7:20
entirely 8:21 321:6	epigenetics 319:3	365:6	187:12,18,19 187:21 188:2,9	119:24 159:10 177:15 231:7
entirety 339:2	epithelial 6:14 24:18 205:8	ethical 224:12 228:4 300:3	190:9,24 192:5 195:8 197:9	270:7 375:11 examining
entities 111:11 111:13	206:8 207:2 259:17,20	ethically 298:2 Ethics 100:7,18	199:4,21 200:6 200:20 205:10	138:10 177:22 198:19 204:12
entitled 5:11 28:9 59:12 60:10,14 271:3 326:12	260:9,21 271:5 282:22 305:23 328:2,13	100:20 101:18 ethnicity 340:21 etiology 107:16	220:19 229:17 242:2,8 244:1 244:11 247:1	225:6 234:6 377:5 example 38:15
entity 308:12	epithelium 31:13	224:2 268:23 273:1	249:11 250:15 253:18 256:3	62:15 77:17 78:7 101:10
entry 16:16 18:23	equally 58:12 equate 171:9	evaluate 151:5 230:4 353:2 354:3	257:18 258:3 268:11 278:18	122:12 123:2 129:23 143:1,1
enumeration 148:7	equipment 308:22	evaluated 209:14 231:1	281:21 284:21 305:13 306:3	143:5,14 144:14 145:1,8
environment 106:12 115:8 164:20 172:13 174:22 175:6 240:4 243:14 320:15,16	equivalent 299:8 320:20 errors 62:20 63:2 64:7,9,20 64:22	287:21 evaluating 5:23 117:7 142:6 181:13 200:15 202:12 210:17	306:15 312:12 314:10 321:1 338:20 349:15 351:10 353:15 355:8 363:15 364:21 365:1	174:8 176:3 177:17,21 240:22 321:12 321:22 324:18 325:12 326:7 340:18
environmental 322:16	Eslick 220:6 Esquire 2:4,5,11 2:15,18,21 3:5 3:9,14	evaluation 8:21 101:15 192:12 205:16 222:6 230:16,20 231:10 234:2 379:9	evidence-supp... 140:20 evidenced 193:15 224:24 276:16 323:17	examples 123:6 168:19 183:18 315:19 316:12 322:12
EOC 328:14	essential 162:19 163:23	event 174:1 189:19 265:4,6 323:14 324:4 324:12	evolution 265:5 ex- 135:23 275:11 361:10	exception 82:4 exceptions 392:4
epidemiologic 33:20	essentially 167:18 212:22 288:9 361:5	events 106:11 264:22	exact 54:7 55:1 56:12 85:2,10 176:9 327:11 347:3	exchange 355:9 exclude 275:12 excluding 380:4 380:5
epidemiological 220:5	establish 140:16 established 116:18 117:21 118:1 140:17 161:23 168:4	evidence 5:23 26:24 31:5,7	exactly 51:23	exclusive 259:19 260:7 339:24
epidemiologist 88:18 288:19 288:22				
epidemiologists 157:23 224:18 257:11				
epidemiology				

exclusively 262:21 336:10	86:17 87:3,15 96:24 97:4,8	25:4 32:9,13 196:14 209:18	244:24 269:5	235:12,15
excuse 20:21	97:19 122:9	281:21 314:10	explanation 49:11 56:3	240:4,8 248:3
27:12 33:13	141:24 142:2	experiments 87:1,7 365:7	361:11,14,15	248:5,23 252:4
55:11 73:5	143:21 144:6	383:9	expose 320:15	252:7 253:15
76:19 79:14	181:6 185:9	expert 4:16 5:9	exposed 56:19	253:16 254:12
88:8 135:10	190:16,19	8:13 40:8,18	57:2 99:22	256:17,21
152:17 158:5	192:23,24	41:11,20 42:15	153:2 156:11	259:9 261:17
167:10 170:7	208:7,10 209:1	43:12,15,20	161:8 200:4	277:12,22
193:24 197:16	218:9,13	44:23 46:9	205:7 206:7	289:24 304:12
225:21 236:12	249:21 250:1	48:1,14 49:1	207:1 253:6	320:16 322:16
250:10 251:21	270:22 271:2	49:20 50:9	258:16 259:14	323:2 324:22
272:12 291:10	275:21 326:17	52:18 54:21	321:8 322:8	325:8 344:24
291:10 302:21	326:18,21	57:13,17 63:12	338:12,23	360:4 361:13
345:4 369:24	327:8 357:12	69:15,18 72:7	340:7,15 344:2	374:9,23
389:10,17	359:14 361:20	74:12 85:15	345:12	375:16
EXECUTED 393:9	367:21,22	93:24 100:14	exposes 159:17	exposures 107:21 119:2
exemplary 143:17	exhibiting 372:8	102:4 114:21	exposure 94:2	226:2 252:8
exercise 210:23	379:16	129:2 134:10	95:9,10 96:5	313:14
exhaustive 364:24	exhibits 4:12	135:24 148:15	96:16 113:2	express 269:10
exhibit 4:13,15	82:23 83:3	181:9 219:22	114:4 121:4	expressed 120:12 389:22
4:17,19,22 5:2	185:12 357:9	223:2 235:5	146:20 150:20	expressing 270:14
5:6,8,10,12,14	exist 116:2	245:7 247:3	154:7 156:1,6	expression 268:4,17
5:16,20,22 6:2	168:9 383:22	256:23,23	156:16,17,21	333:14
6:4,6,8,10,12	existence 44:7	257:10 266:1	156:24 157:7	extend 235:2
6:16,18 14:9	exists 205:2	285:22 288:3	157:13 159:8	extended 283:2
14:11,22 16:4	318:15	288:13 295:3	160:4 162:3,3	extending 210:6
16:7 19:4,7	exogenous 323:1	296:10,16	162:5,7,15	232:24 252:3
20:4,9 21:5,14	exogenously 318:1	297:7 352:17	163:3,4,11,13	extends 108:24
21:18,24 22:8	exome 310:7	385:15	163:15 164:18	extensive 79:5
22:11,14,16,19	expand 365:22	expertise 371:24	164:21 165:5,7	exterior 304:15
22:24 23:3,7	expanded 46:7	experts 39:23	165:15,19,20	external 314:24
23:13,16,18,22	85:23 87:8	40:4 52:24	166:2,5,9	extracted 213:17
24:1,15 30:12	373:2	127:21 201:1	176:22 177:6	extramural 109:16
30:13 33:6,7	expect 257:17	233:11 254:16	177:24 178:10	extramurally 300:5
38:4 39:18	258:2,14,18	262:10 295:16	178:24 179:10	extrapolating 210:12 236:8
48:2,4,14 49:2	259:7,13	295:23 296:1	183:8 196:15	285:12
49:20,22 50:9	expected 268:23	354:6 383:24	199:7,12	extrapolation 214:7
59:7,11,23	270:18 273:2	explain 70:12	204:14 206:2,6	
60:2,13 72:2,3	experience 10:9	94:16 118:7	211:14 214:13	
75:12,14,22,24	109:23 119:10	204:6 238:13	218:2 224:8	
76:6 82:19	366:19	278:11 321:6	226:5,7,10,12	
83:4,4,23 84:3	experiences 169:14	360:9	228:22,23	
84:5,8,10,11	experimental	explained 268:17 315:21	229:20,23	
		explaining	230:5,14,18	
			233:7 234:18	

F	22:19 23:19	341:20 348:3,5	332:12,22	371:5 372:2
F 3:13 391:1	28:15,17 32:3	fascinating	334:10,16	Finally 281:18
facility 104:18	38:5 54:21	97:22 154:13	335:19 336:24	305:11
fact 74:6 79:7	85:12 88:5,18	198:18	337:23 340:16	financial 100:10
103:4 106:5	89:13 91:14	fashion 57:15	341:10,22	100:18,20,22
130:3 135:22	95:2,16 99:11	115:15 143:18	342:8,15 343:2	101:18
154:20 173:17	99:12 104:3	228:2 374:22	345:9,18 347:6	find 32:22 37:24
175:13 181:12	109:4 161:19	fat 362:19	349:18 350:11	53:22 63:17
188:8 197:8	163:21,22	FDA 6:5 111:7	351:17 353:6	66:6 68:21
202:13 204:22	189:10 210:13	161:24 192:15	354:8,14 355:7	69:4 143:4
215:15 219:17	214:3,3 217:19	193:11,16,18	355:17 356:4	235:5 257:1
252:6 265:24	218:5 251:19	194:11,15	356:10 389:7	273:17,24
268:10 269:23	263:20 299:18	195:11,15,20	fiber 129:9	288:5 304:7
271:8 273:17	306:21 312:10	196:1,11 197:2	fibers 129:20	339:5 363:15
274:5 276:18	312:11 342:5	197:8 200:7,9	235:7	368:14,20
294:7 298:11	357:22 361:2	200:23 202:8	fibroid 368:21	378:3 380:9
300:17 305:7	379:5 381:16	203:4 204:5,10	fibrosis 30:24	383:12
320:4 323:14	383:8 384:5	204:17 205:2	250:17 363:1	FINDEIS 2:11
358:13 364:13	388:2	205:19 206:4,5	363:10	finding 120:22
370:18 382:20	fairly 100:24	206:14,17,22	fibrous 305:5	141:13 250:3
factor 114:18	108:22 162:8	226:24 305:8	355:24 371:3	271:20 275:5
260:24 286:18	338:2 365:7	357:12 359:1,7	field 116:24	279:19,23
factors 77:19	fall 46:5 297:6	373:11,17	154:13 234:12	280:10 281:6
78:10 107:21	fallopian 199:23	384:16	fields 284:13	281:12 284:20
110:24 172:2,2	304:16 305:22	FDA's 192:11	Fifteen 191:6	304:23 384:2
276:7 277:5	306:2,5	192:18 200:8	Fifth 2:17	384:20
319:8 343:1	familiar 10:8	201:24 202:12	figure 159:3	findings 29:23
369:11	49:5 75:9 89:6	203:12,22	212:2,3,5,5	173:10 188:16
facts 106:4	89:12 96:16	204:9 357:20	213:3 214:24	188:17 192:2
167:13 168:1,5	100:8,10 103:1	358:2 372:9	232:8 360:3,9	192:11 195:6
168:6 327:23	109:19 124:22	feature 350:24	360:10,12	210:7,11 220:9
329:7,20	173:10 192:20	features 328:17	361:9,10	254:22 282:4
factual 74:9	193:9 249:19	federal 29:16,20	figures 211:20	282:15,19
79:10 81:5	306:6 375:21	291:22	212:5	371:1 374:13
140:17 168:19	family 345:21	feel 190:13	file 40:24 42:5,6	375:20 383:7
329:21 331:5	346:6,11,14,21	241:8 313:13	44:7	383:10 388:1
factually 162:14	346:23	female 192:7	files 13:11,12,14	fine 42:8 170:5
faculty 89:8	far 13:11 48:21	Ferguson 3:5	filing 373:3	194:2 237:3
98:4,5,14	92:21 121:19	4:5 301:21	final 21:24	352:1
101:12 103:9	126:2 152:11	303:13 306:23	45:14 46:17	finish 79:23
103:24 310:21	179:3 183:9	306:24 307:3,5	47:7,11,12	152:17 158:11
312:14	186:17 198:11	314:8 317:3	63:21 87:21	158:17 197:16
fail 77:24 78:11	198:19 199:24	320:2 321:4	272:24 369:23	236:16,18
failed 373:5	214:6 234:12	322:4 325:10	finalized 45:21	274:20 292:17
fair 10:6,10,11	263:10 284:7	326:8,20 328:5	45:23 47:4	finished 194:1,1
18:13,16 20:4	299:15 312:7	328:8 329:9,14	97:13	197:17 202:23
	318:4,24	330:3 332:3,6	finalizing 46:8	274:19

finishing 301:20	Focusing 274:22	87:10,17 88:8	215:19 216:5	329:5,12,17
first 7:18 10:9	folks 64:14	89:16 90:6	216:24 217:21	332:21 333:2
16:16,20 17:1	89:22 173:4	91:16,22 92:17	220:13 221:7	333:23 335:15
17:13 19:18	195:11	93:8,22 94:14	222:22 223:19	336:4 337:16
20:5 22:11,16	follow 146:1	95:4 97:11	225:3,17	340:12 341:15
23:16 31:5	follow-up 357:3	103:19 104:7	226:21 227:22	342:3,23 345:4
51:2,11 60:22	389:5	105:13 107:19	228:16 229:14	345:15 346:20
61:11 72:7	followed 173:4	110:16 113:8	230:7 231:4,16	348:8 349:13
75:23 76:7,22	173:19	113:19 116:8	232:12 235:21	350:8 351:7,13
77:1 84:15	following 174:2	116:11 117:12	238:17 239:7	352:14 354:2
98:2 116:22	266:12 294:23	118:4,21 120:9	240:12,21	354:11,23
142:9 168:8	328:17 329:20	122:6 124:1,4	241:21 242:16	355:12,22,24
184:9 189:18	330:4 392:4	125:2,24	243:22 245:4	357:24 358:10
189:19 191:22	follows 7:21	126:16,22	246:24 247:10	359:5 360:1
191:22 192:4	53:13 55:6	127:16 128:23	248:8,18 250:6	361:7 362:8
193:6 221:10	footnote 74:4	130:11,23	250:10,22	363:3 364:17
221:18 226:22	foregoing 391:4	131:16 133:4	251:7,21	365:16 367:2
239:20 246:21	391:8 393:4,7	134:8 135:10	252:15 253:10	369:19 370:10
252:22 264:1	foreign 239:10	135:13 136:12	254:6 255:10	370:21 371:8
285:4 307:22	239:10 248:14	137:5 138:18	255:20 257:21	373:14,22
328:24 330:5	250:16 254:23	143:7 144:2,13	258:6,20 261:7	374:5 375:8
362:22 366:4	257:2,19,24	144:24 145:19	261:14 262:5	376:3,18 377:3
366:10 368:12	258:15 266:14	146:8,18	263:22 264:24	377:15 378:6,8
fit 266:16	267:20 362:8	147:17 148:1	266:4 267:1,13	378:10 379:7
267:22	362:14,16	148:20 149:5	269:2 270:3	379:19 380:12
five 236:23	foreign-body---	150:17 151:19	271:17 275:20	380:20 382:12
237:2 309:9	205:6	153:10 155:4	276:13 279:10	383:1 384:10
311:9 362:9,17	form 13:6 22:3	155:15 157:3	280:17,21	385:1,20 386:8
387:24	25:22 27:21	157:17 159:5	281:9 282:7	388:4
fix 79:17	29:16 31:21	159:21 160:12	283:20 285:17	formal 9:24 85:4
flag 50:3	32:5 34:16	161:12 162:24	286:12 287:3	87:8 205:16
flaws 194:17	36:5,15,24	164:13 169:17	287:23 289:1	227:24 233:14
195:21 196:1,7	37:12 43:23	170:17 173:15	289:12,17	295:5,7
197:2,18	45:7 46:12,19	174:20 175:18	290:21,24	formation
Fletcher 84:15	48:10,16 49:15	177:10 178:8	291:3 293:14	173:24 242:21
87:6,12	52:13 54:14	178:22 180:5	294:5,19	348:2
Flip 60:7	57:19 58:1	183:23 185:22	295:19 296:4,9	formed 179:24
FLOM 2:20	59:3 61:3,9	188:5 189:12	296:12,19	forming 18:14
FLW 1:7	62:13 63:9	190:12 191:2	298:15 299:1	24:21 44:23
focus 88:20 99:3	65:5,23 66:2	191:18 192:14	302:21 303:2,8	110:2 111:5
114:14 156:3	66:16 67:2,10	195:23 199:10	303:8,20	121:17 129:13
197:13 228:20	67:22 68:13	200:17 201:6	304:18 305:2	146:13 147:20
231:5,7 232:22	69:2,7 70:10	202:5 203:18	306:9,17	173:3 176:13
233:5 277:3	71:8 73:2,11	205:21 206:21	310:11 314:1	199:5 250:8
301:3	74:17 78:18,24	207:9 209:5,16	316:21 318:11	291:23 374:13
focused 8:21	80:6,13 81:1	210:15 213:13	320:13 321:10	384:21
353:4	81:21 86:10	214:21 215:10	324:24 325:21	forms 168:10

found 17:20 30:20 122:20 124:16 144:5 159:23 160:1,2 161:2 162:4,16 176:4 187:12 188:2 202:8 204:2 219:11 221:4 252:12 271:12 272:8 273:11,15,20 274:8,23 301:4 306:5 319:4 378:19	30:19 68:3 141:22 312:22 357:9 full 31:5 33:13 33:19 39:4 51:2 72:7,20 76:7,22 77:2 103:23 112:11 121:22 122:1,1 203:3 205:1 243:2 274:14 330:5 function 62:21 63:3 functionally 299:7 343:11 functions 61:19 62:6 fund 109:8 fund- 65:6 fundamental 52:22 53:7 57:12 61:12 62:16 65:13 68:17 74:9 79:11 81:6 91:18 106:4 173:18 183:15 185:6 226:1 233:6 329:7,19 348:15,16 fundamentally 105:5 174:8 185:1 217:5 263:12 294:8 fundamentals 74:13 168:14 funded 108:17 109:3 296:24 297:11 300:5 funding 89:9 90:21,22 98:12 98:12 109:7 298:7 299:4,11 300:2,5 312:3 funning 300:10 further 160:6,6	162:18 203:1 306:22 322:5 326:11 328:1 363:8 369:1 372:5 379:9 389:3,8 390:5 391:11 furthermore 190:2 197:1 future 97:24 <hr/> G G 392:1 gain 319:20 Gates 182:22 183:14 208:6 GC1a 360:21 GCA1 360:21 gene 51:13,13 51:20 53:9 55:7,14,20 56:17,24 60:10 64:9,21 217:15 260:2 313:8 321:23 322:6 322:14,20,22 324:20 325:6 326:2 333:13 333:14 334:6 335:18 336:17 346:17 gene-dependent 321:12 general 70:17 101:9 102:3 105:16 106:21 113:1 115:19 116:21 117:6,9 118:9 152:11 169:21 170:18 171:3 199:3 202:6 210:5 217:7 224:4 259:4 316:2 323:3 337:12 338:1 355:23 363:24 365:20	371:21,22 generalizations 172:18 generalizing 172:15 generally 8:19 70:22 167:15 175:12,13 207:13 243:17 263:18 286:24 297:3 311:5 323:15 330:7 330:18 336:9 344:15 349:2 generate 218:1 354:16 generated 215:8 generation 182:1,9 200:11 209:9 211:4,23 212:7,21 214:12 215:17 216:3 236:7 318:7 genes 61:18 62:4 64:7,19 217:10 310:9 321:22 329:23 331:2 331:17 332:15 336:7 338:9 339:20 340:22 346:4 347:11 347:18,23 348:6,16,20 genetic 51:3 53:14 54:2 56:10 62:2 106:8 320:5,23 323:21 328:17 329:22 330:8 331:1,1 345:23 346:13 347:1 347:10 348:2 348:11 genetically 213:23 214:5 216:20	geneticist 119:10 genetics 88:17 318:17 320:15 326:13 345:20 346:23 348:14 genital 265:17 267:15 genome 73:3,13 88:21,23 309:18,21 343:17 genome-wide 343:13 genomic 8:22 308:1,11 309:5 genotoxic 354:20 355:5 genotoxicity 355:1 geographic 198:15 germane 65:10 252:5 getting 38:3 152:23 233:22 242:4 270:5 297:22 321:7 336:9,20 389:20 giant 250:16 give 19:20 27:16 38:14 122:4 124:17 144:14 184:19 214:17 215:7,16 216:2 284:16 303:24 326:6,7 351:24 368:16 369:24 given 9:11,16 32:9 33:3 68:16 90:17 92:13 103:24 123:20 140:5 152:20 163:9 172:6,7 174:4 180:13 192:5
--	--	---	--	--

234:21 246:11	208:10 219:5	growth/survival	59:15 135:21	363:21
258:10 259:4	236:15 312:24	77:19 78:9	249:20 301:20	Heller 6:11
284:18 293:10	312:24 357:5	guarantee	303:12 306:22	249:16 250:13
299:17 301:9	good 7:24 8:1	338:10	handed 16:9	252:11 254:22
321:13 327:18	79:16 267:7	guaranteed	19:6	378:2,11 379:5
347:16 348:12	307:4 312:7	321:14	handing 20:19	379:12 380:8
377:23 389:15	321:22 341:20	guess 109:22	21:17 22:23	Heller's 249:12
391:10	Google 121:10	119:23 253:3	33:4 50:8	250:1,3
giving 106:15	GORDON 3:3	255:2,2 326:17	59:10 75:11	help 47:10 232:7
187:7	gosh 9:2	335:21 362:11	82:18 218:12	240:4
glutathione	GOTSHAL	383:17	249:24 271:1	helped 201:16
183:15	2:14,17	gynecologic	handy 192:22	helpful 83:7
go 9:5 53:19	government	327:2,3 370:3	happen 255:17	135:5 140:24
55:11 56:23	4:20,23 19:13	gynecological	happened	141:2 198:6
61:15 67:24	21:21,21 90:20	340:3	333:19,19	229:2
75:5 120:22	90:22 300:17	gynecologist	happens 251:4	helps 127:23
139:8 140:19	grade 196:13	370:3	happy 10:24	385:9
141:13 166:13	197:21 328:19	<hr/> H <hr/>	hard 13:21,23	Henderson
171:24 236:21	330:7,18	half 98:22	30:16 44:3	304:4
238:7,13 260:2	grant 108:18,23	309:10	Harper 182:22	hereditary
267:19 290:18	109:5 300:14	halfway 266:12	241:11	345:5
301:15 313:1	300:15	hallmark	head 43:18	heterogeneity
313:13 327:24	granted 312:9	316:18	123:24 347:22	258:11
330:14 338:7	grants 91:3	Halperin 2:21	heading 327:12	high 322:2
339:11 343:3	98:13 300:18	Hamilton 5:7	heal 77:24 78:11	323:22 328:19
348:5	granulocytes	25:3,16,18	health 5:24	high- 322:23
goes 221:1 291:2	316:6	26:3,9,14 27:8	107:11 108:21	higher 183:16
299:7 331:16	granulomas	27:8,14,19	109:11,13,15	212:21 260:5
332:15	362:9,16	29:23,24 30:18	110:1 111:7	335:2 336:22
going 28:10	graph 360:12	31:19,22 32:1	142:8 258:12	348:1
41:19 55:4	great 42:24	32:6,17,23	384:16	higher-risk
56:14 79:20	237:7 263:17	34:6,12,22	healthcare	336:17
82:11,11	288:5	35:4,7,11,14	308:7 309:14	highest 361:12
152:18 158:5	greater 148:9	181:21,21	healthy 317:11	highly 105:8
161:6 166:17	161:23 192:10	182:16 185:7	heard 17:1	hired 8:16 91:10
177:2 236:12	345:11	185:17 186:2,4	hearing 307:12	93:3 95:15
237:9 259:12	greatest 361:4	186:7,9,22	391:10	96:7,17 102:14
265:1 301:19	ground 10:7	187:9,11,16	heavy 127:14,22	histologic
302:3 307:10	group 58:5	188:16,18,21	133:7,11,18	259:17
309:8 339:2	87:12 89:8	189:7,14	136:9,21 137:3	histological
349:19 356:16	180:10 192:9	191:10 207:19	137:14 154:2	329:8,20
356:19 357:8	200:5 323:20	361:20,23	234:18 352:21	378:23
Golkow 7:4	grow 61:20 62:7	362:2 374:13	353:7,12,16,18	histopathologi...
gonna 14:8	106:13	Hamilton's 35:1	380:21 381:3	259:2
28:19 39:2	growth 77:20	hand 14:8 16:11	381:15 382:3	historical 371:1
41:18,18,22	78:10 106:2	19:3 20:17	held 1:15 7:7	historically
42:13 64:4	348:5		297:4 310:18	177:16

history 284:11 284:11 345:22 346:6,11,14,23 368:21 383:17	227:6 249:9 253:5 254:3 365:9 376:11 379:22,23,24 380:7,8,9	82:6 327:20,21 329:2 332:19 IDENTIFICA... 14:12 16:5 19:8 21:15 23:4 30:14 33:8 49:23 59:8 72:4 75:15 82:24 142:3 190:17 193:1 208:8 218:10 249:22 270:23 326:19 367:23	117:15 216:16 important 52:15 57:10 114:24 115:1 137:2,8 191:7 215:2,22 248:1 266:10 274:12,20 298:6 300:1 319:9 346:24 348:18 351:1 importantly 197:2 201:7 215:20 269:4 272:3 274:11 277:9 308:10 impossible 217:1 in-depth 365:7 Inability 344:18 inadequate 110:12 inappropriate 106:15 114:14 256:19 259:5 inarguable 129:19 162:1 incessant 351:3 351:6 incidence 268:22 incidences 192:7 include 45:19 69:23 92:2 124:7 125:11 139:12 146:22 148:17 168:8 178:10 200:12 219:24 220:4 310:6 326:1 341:6 359:8 365:11 383:17 included 36:7 45:12 73:21,22 83:13 85:23 87:11 91:23 118:15 126:6 129:6,6 138:15	139:10 146:19 146:21 148:21 150:5 157:20 195:17 205:13 212:9 226:23 254:10 289:4 347:2 352:6 358:7 387:8 includes 86:1 137:10 153:22 328:14 including 19:2 71:6 78:15 79:11 91:9 92:23 105:21 107:24 116:21 118:9 119:8 138:11 168:10 201:16 206:3 219:21 234:16 236:2 263:5,8 263:15 279:14 314:4 320:16 352:12,20 388:8 inclusion 31:2 188:21 197:20 274:13 385:2 386:23 incomplete 155:4 189:17 204:22 257:21 258:6 269:4 inconsistent 268:11 incorrect 265:11 359:3 increase 160:5 211:3,17 212:19 214:12 214:14 238:3 238:22 239:22 275:4 314:3 324:5 336:8 337:12,13,22 338:22 345:11 349:3 362:7
hoc 101:19				
hold 21:3 115:9 138:3 145:15 189:23 286:8 363:18 364:6 388:20,23 390:1	human-based 375:13 humans 166:7 185:1 210:12 351:10 364:21 365:3,13 374:19,22,24 375:6 376:7			
holds 288:14	hundred 338:4			
hopefully 308:19 349:21	hundreds 100:24	identified 55:23 82:20 121:22 156:20 159:9 185:8 187:9 190:7 207:19 207:22 249:17 275:4 323:21 347:18 388:19		
hormones 313:15	Huntsville 1:16 1:17 7:8	identifies 200:10 387:15		
hour 79:20 158:6 236:13	hypothesis 32:18 36:1 116:7 235:9 289:21 294:3,9	identify 11:8 19:10 23:6 24:11 83:7 193:20 301:7 348:14 373:5		
hours 17:23 18:14 19:2 47:14,18,20,23	hypothesized 278:15,16	Illinois 3:9		
HudsonAlpha 88:21 89:2,3 89:18,22,23 98:1,3,8 100:7 100:11 101:22 101:23 102:6 102:12,20,24 103:11,16 109:4,9 287:15 299:13 307:24 308:3,13,16 310:22 311:13 311:23 312:6 312:16	hypothetical 155:5 166:12 257:22 258:7 294:14	illustrate 106:7 114:15 211:2 324:2		
	I	illustrated 314:6		
	IARC 305:3 366:22 367:3 367:10	Imerys 3:2 307:5,6		
	IARC's 304:23	immune 34:3,11 34:24 35:5,9 35:10,11 181:22 185:6 188:20,23 240:2 244:9 248:16 251:1 350:24		
	idea 17:19	impact 130:21		
	identical 51:19 54:20 55:17 56:1 57:7,17 57:20 58:9 61:24 63:2,13 65:2,16,20,24 66:6,7,13,22 67:8,18 68:21 69:4 73:18 74:5,15,20 78:16,22 80:3 80:11,18,19	impairs 344:9		
HudsonAlpha's 100:18 101:12		importance		
Huh-uh 41:17				
Hulfish 2:14				
human 128:8 182:12,13 184:23 190:3,5 196:15 201:2 209:20,23 210:8 214:7 217:15 218:6 222:5 225:8				

increased 5:18	198:20 318:18	114:22 115:2,6	235:4 239:5,15	129:22,24
83:11 84:14	320:19 325:4,7	115:14,15,21	239:19 240:7	130:4 136:20
160:3 166:2	345:7,24 353:2	116:6,7,17,20	241:2 243:19	154:22 163:10
182:9 212:23	353:3 386:21	116:24 117:4,9	244:19 247:12	165:8,18,20,22
254:12 256:16	individually	117:15,20	248:4,6,13	168:15 169:7
265:17 267:16	58:4 67:4,13	118:1,8,9,10	252:1,2,4	169:11 170:24
273:3 275:1	128:15 174:11	118:19 119:1	258:3 260:10	171:19 172:24
276:20 278:11	277:17	119:20 121:4	260:20,23	174:12 175:7
279:16 321:3	individuals	128:4,12	261:4,5,12,22	177:23 178:9
322:23 336:14	114:10 156:6	129:21 130:2	261:24 262:3	178:11,18
337:10 344:1	174:23 258:11	135:19 136:6	262:14,19,24	188:20,23
344:23 346:18	258:16 259:14	137:23 140:8	263:3,11,19	189:4,8 192:4
347:19 369:8	284:5 319:6	145:17 146:6	264:5,20	197:5 204:13
369:10	323:22 324:5	146:14 147:13	266:13 267:19	205:7 206:2,7
increases 110:13	334:20 347:9	147:21 149:3	268:24 269:19	207:1 209:9
181:24 338:11	348:11,13	149:16 150:15	270:8 271:4,23	211:15,16
340:6,14 345:6	induce 188:13	150:22 151:12	272:20,24	230:17 233:8,8
increasing 160:3	induced 20:12	151:17,24	275:13,15	238:8,23
160:3 211:18	179:17 363:1	153:8,16,18	276:2,8,10,16	239:11 240:2
233:19	induces 167:3	155:1,10,19	276:18,22	240:15 241:17
ind- 233:16	178:11,18	156:1 157:8,14	277:16,21,24	242:1,9,12,20
independent	191:14 350:5	159:1,8,18	278:4,5,6,9,17	243:10,13
115:12 229:19	350:18 364:14	161:10 162:20	279:7 281:24	245:2,19,22
230:14 246:3	inducing 350:5	164:2,6,10,18	282:23 283:18	246:1,6,7,12
278:1 295:13	350:17	167:24 168:3,9	283:24 284:2	247:8,17,18
325:3 385:5	industrial 126:8	169:1,2,8,13	284:12 285:6	249:10,15
388:1	inert 255:8	169:14 170:14	285:10,15,24	250:20 251:15
independently	inevitable 318:8	170:15,20	293:19 305:14	252:13 253:7
288:8	infer 256:19	171:4,7,10,12	306:15 313:16	253:17,24
indicate 214:11	inflamm-	171:18 172:8	316:16,18,24	255:13,14,21
274:9 277:15	241:17	172:11,12	349:3,4,9,16	258:10 259:1
322:6 327:1	inflamma-	173:13 175:10	349:22 350:3,5	259:10 260:13
347:8	381:3	176:17 177:14	350:17 351:3,7	260:16 261:19
indicated	inflammation	177:17 178:4,5	351:11 353:12	264:10,16
281:16 335:6	6:13 20:11	179:1,1,11,17	353:14,16	265:9 268:7
indicating	21:6 24:3,16	180:1,16	358:21 362:7	270:6 271:13
337:22	24:17 25:19	181:18 182:18	362:17 363:15	271:21 272:8
indicative	26:4,7,11,15	182:19 183:8	363:19,22	274:4 275:11
256:20	26:17 27:9	183:20 184:12	364:6,14,21	275:12 276:16
indicator 345:22	29:24 32:2,19	184:15 186:24	365:3,13 366:2	276:21 277:2
346:12,15	32:23 36:1,21	188:14 191:15	366:10,11,12	278:18 281:22
indirect 167:20	37:9 91:24	191:21 207:6	367:9 376:7	282:17,21
314:24 348:2	92:10,14,22	207:14,18	379:11 380:17	283:3 284:6,21
indirectly 240:3	93:5,13,14	208:15 209:3	381:3,16 387:4	289:5 315:2,23
individual 61:18	94:3 102:8	211:12 219:8	inflammatory	316:2,13 365:8
62:4 159:17	106:13 114:9	226:18 227:3,5	37:17 77:19	367:13 374:18
177:1 179:4,4	114:13,16,22	229:6 230:23	78:9 92:3	375:5,24 376:5

376:10,15,21 377:12,22,23 378:18 379:2 379:17 380:10 380:23 381:10 385:8 387:12 388:15 influence 326:1 influenced 277:6 369:7 info 113:5 infor 118:10 inform 20:6 22:20 23:18,20 39:11 84:5 85:14,21 180:20 inform- 65:9 information 20:10 21:5 22:5 24:1,15 24:20 27:5 56:8 58:8 65:7 65:13 66:9 67:19 68:14 70:18 71:5,15 74:9,10 75:4 75:18 81:6 85:22 86:2,9 87:2 97:19 100:4 110:23 111:10 112:4 119:22 120:3 123:6,11,13 124:1,6,9 125:1 127:20 131:19 133:22 135:20 136:2 136:16 139:2 140:3 149:11 151:3 155:6,12 168:19,21 180:9 204:13 205:17 220:5 223:22 234:7 245:6,23 286:5 331:1,5 333:8	333:24 334:5,6 341:8 366:1 371:4 373:10 387:7 388:7 informatory 65:9 informed 83:20 informing 83:5 infrequent 104:23 inhalation 162:3 200:2 226:7 inhaled 199:19 200:5 inherit 334:20 inheritance 347:10 inherited 51:8 51:12,19 53:9 53:14 54:2 56:17,24 321:6 322:6,13 324:19 326:2 335:17 342:17 345:21 346:4 346:17 347:17 inhibition 270:19 initial 47:16,17 177:14 243:12 248:15 249:2 265:6,8 293:16 327:19 348:17 initially 47:20 initiate 242:13 243:20 253:8 initiated 77:17 78:7 initiates 239:15 initiating 265:4 initiation 117:1 117:16 118:11 156:1 204:15 260:17 262:22 263:6 306:1 348:17 injected 31:13	172:20 173:11 185:19 186:5 187:13 188:2 362:9,18 injury 163:10 171:20 238:22 317:12 insert 15:4 inside 61:16 251:14 317:8 317:17 insight 75:17 instigate 239:11 Institute 98:10 108:7,18 109:2 307:24 institutes 99:6 109:15 institution 98:6 98:9,15 101:9 101:21 299:12 299:17 300:16 312:18 institutions 104:1 instruct 44:14 292:15,24 instruction 63:2 293:1 instructions 61:19 62:5,20 insufficient 193:19 244:11 insult 78:6 171:20 344:7 insulting 235:8 intact 318:6 integration 95:22 integrity 318:5 intending 224:22 intensity 233:1 interest 299:14 299:16,21 interested 391:13	interesting 58:12 106:20 124:23 198:15 212:4 interestingly 223:20 interpret 213:1 interrelated 348:8 interrupt 272:13 interval 32:7 279:18 280:6 280:14 281:3 359:23 intramural 109:17 introduce 52:21 327:23 introduced 318:1 introducing 331:5 introduction 68:17 introductory 56:8 57:12 62:16 65:7 79:5 330:17 invasive 275:2 inversely 268:22 investigate 105:7 107:12 213:21,24 investigated 91:12 203:13 investigation 106:19 202:13 298:22 299:2 investigator 98:4 investigators 109:9 196:12 200:10 250:15 invoice 16:17 invoices 4:18 15:18 16:8,12	17:23 18:3,18 102:14 involve 103:24 involved 91:7 347:11 348:16 363:17 364:5 365:23 366:3 372:13 involvement 16:21 89:17 310:10 involves 99:5 involving 104:12 128:8 175:24 299:9 304:8 iPad 11:10 irrelevant 90:9 irritants 387:3 388:13 ISI 121:10 isolation 96:12 IS RTP/FDA 201:1 issue 342:5 381:12 issues 8:20 147:4 it'd 217:5 It'll 135:4 Italian 124:24 125:9 items 11:7 13:2 330:8,21 <hr/> J <hr/> J 2:11 3:5 372:14,14 J.D 2:8 Jackson 2:7 JAMES 3:9 James.mizgal... 3:10 January 1:11 7:5 16:10 265:22 373:4 Jennifer 2:5
--	---	--	--	---

375:5	357:15,16,20	224:19	listing 43:14	373:11 375:11
leads 26:11 32:2	359:3,7 372:20	limita- 290:19	333:4,24	381:2,19,20
36:1,12 37:9	level 154:7,9	limitation	lists 386:23	382:18,23
114:1 129:17	155:18 172:8	160:13 189:14	literature 12:23	384:11 385:22
170:15 174:18	232:19 243:8	212:14 276:14	12:24 37:1	literature-cited
178:6 209:3	248:19 308:19	292:2 293:10	39:4 46:7,21	122:20
282:17 315:2	319:11 360:4	377:19,20	48:18,20 50:13	litigation 1:6 7:4
380:17 381:4	levels 258:12	limitations	50:14 69:21	7:11 8:12 17:3
learned 352:19	Levy 1:14 4:16	32:13 186:13	93:18 95:6,8	54:21 63:13
leaving 311:17	4:18 7:12,17	186:15 190:4	96:13,19	69:19 91:11
312:13	7:24 8:5 15:20	210:3 214:6	114:19 119:9	181:10 187:8
Leavy 204:16	18:8 29:9,22	290:17 293:2	119:21 120:17	257:1,6 266:2
led 29:24 32:23	32:18 44:9	limited 9:21	120:18 121:2,6	296:2 298:13
34:13 35:12	72:6 88:11	115:17 127:8	121:14,16	363:18 364:5
374:24 375:11	93:16 110:3	139:16 156:9	122:13 123:4,8	little 152:24
376:16 377:12	111:6,14 116:6	165:17 167:16	124:8 125:7,12	158:9 168:12
left 56:16 182:7	117:8 122:21	175:1 186:15	128:6 132:23	236:13,15
Leigh 2:4 16:24	125:15 158:14	224:9 225:4,11	133:21 138:20	238:14 298:21
17:2 332:7	170:9 179:22	230:24 261:10	139:15 143:4	307:16,23
Leigh.odell@...	183:20 203:23	266:23	144:6,8 145:10	310:12 324:15
2:5	204:16 260:19	Lin 272:3	148:3 149:6	327:24 346:9
lend 385:10	272:15 291:6	line 24:14	152:9 154:12	368:13
length 228:18	292:12 307:4	167:13 337:1	159:7 160:5	live 322:3
230:19 247:16	357:3 359:11	linear 160:21	161:21 166:4	lives 227:10
248:2,4	362:22 363:18	lines 210:3,4	167:14,15	LLP 2:7,14,17
lesions 305:13	368:9 369:23	215:11 247:24	173:17 174:14	2:20 3:3,8,12
305:16,19	372:8 374:17	320:24 323:9	175:21 177:15	LNP 275:2
306:4,6,13	376:12 387:22	331:23,24	178:15 193:21	local 238:22
362:18	389:19,21	360:16,21,23	202:9,14,20	location 232:20
lesser 148:9	392:3,22	361:1 376:6	203:6,13 204:2	logical 199:11
let's 12:11,14,14	393:16	380:5	204:11 207:13	Lois 1:19 3:21
15:24 49:20	Levy's 77:9	link 221:11,23	220:1 242:6	7:15 391:18
50:20 53:19	LHG 1:7	222:7	252:6 255:13	long 161:22
55:4,11 56:14	Liability 1:6	linked 179:11	256:3 279:1	166:11 224:11
56:23 59:22	7:10	278:16	286:15 289:22	228:14 233:1
61:15 63:20	libraries 121:23	linking 278:18	290:10 293:17	303:8 319:13
71:23 75:5	lies 363:12	284:21	293:18 294:12	longer 106:21
82:9 86:22	life 318:13	Lisa 75:7	295:1 305:16	158:10 191:10
141:24 147:19	lifestyle 313:14	list 12:24 13:10	314:12 325:12	212:23
169:23 170:2	318:17 319:8	39:4 40:8	331:7 334:2	longest 359:22
197:13 272:7	319:20 320:16	122:9,13,20,22	350:23 355:14	Longo 40:13,15
326:11 334:13	lifetime 227:12	330:20 348:19	356:5,8 357:21	40:20 41:5,9
342:1 343:3,20	234:5 254:12	387:23	358:3,6 359:2	41:12 42:17
348:22 352:2	324:5 337:2,19	listed 41:7 84:16	359:8 364:2,11	43:1,8 45:4,9
letter 83:13,18	lifted 48:8,11	138:7 330:9	364:15 365:1	45:11,14,20,21
84:8 194:11	light 200:24	387:2,3	365:24 366:4	46:8,16 47:2,4
195:16 357:12	likelihood 131:7	listen 135:4	367:7 373:2,6	47:8 130:7

370:7 383:4,7 383:12 384:2,6 384:8,21 385:16 Longo's 45:24 370:12,18,24 longstanding 161:22 look 20:14,16,21 20:24 29:2 30:6 38:1 50:20 53:12 64:7,19 71:23 75:1 110:3 122:8 123:22 124:13 128:11 145:6 160:17 160:20,22,24 175:21 177:15 186:16 204:24 214:24 221:9 229:4 236:3 248:13 256:5 266:8 272:7,9 276:7 277:4,9 301:16 302:12 313:5 325:14 326:11,15,24 327:10 328:23 330:4,15 331:15,20 334:13 337:9 339:16 343:20 348:10,22 350:19 351:19 355:15 357:19 358:5 360:8 362:21 369:1 372:19 386:9 looked 32:6 67:17 68:4 69:18,20,20 128:14 146:20 152:8 160:9 166:4 173:2 188:1 200:5 224:10 226:7	249:16 256:24 277:1 284:5 297:20 306:12 306:13 355:1 376:5,7 384:7 384:15 looking 24:8,13 29:10 30:5,18 31:4 33:17 42:16 58:17 60:20 63:23 110:23 132:7 179:14 181:19 181:24 182:11 202:24 213:14 233:21 252:16 265:15 273:21 280:4 293:17 295:24 313:7 325:11 338:19 348:1 351:23 362:4,11 368:15 looks 16:16 17:22 18:2 234:3 loop 303:11 Loss 64:1 lot 361:21 love 158:6 low 268:17 328:18 330:7 330:18 338:2,6 lowering 270:7 LUNCH 166:19 lung 4:24 21:22 106:22,23 165:19 176:5 176:18 234:13 Lynch 343:21 343:24 344:9 344:23 345:2,5 345:10,13 <hr/> M <hr/> M 2:11,15 M.D 88:14	machine 391:5 macrophages 191:23 192:6 264:14 348:24 349:3,11 magnitude 236:7 247:21 248:5,20,24 258:15 259:11 361:4 major 99:3 275:16 276:2 281:24 284:3 304:9 317:7 327:2 majority 98:12 123:12 127:9 231:6 266:1 300:13 387:1 making 52:4 118:24 235:9,9 244:16 267:23 267:24 319:10 345:16 393:7 malignancy 243:15 327:3 malignant 114:2 115:10 169:5 169:10 243:15 328:14 managed 299:21 management 98:17 manages 299:16 MANGES 2:14 2:17 manner 52:22 140:21,24 MANSUKHA... 3:3 manu- 25:23 manuscript 5:3 5:17 23:8,9 25:24 83:6,8 83:18 84:19,23 85:5,13,14,23 86:2,5 87:8	302:13 303:4 363:6 manuscripts 370:15 376:9 maritime 213:17 mark 16:7 30:11 33:5 49:20 72:1 76:1 135:1 141:24 190:19 192:23 208:10 326:16 326:17 360:11 marked 14:9,12 14:22 16:5 19:4,8 21:15 21:17 22:23 23:4 30:14 33:8 38:4 48:2 49:1,23 50:8 59:8,10,23 72:4 75:11,15 76:5 82:19,24 86:16 87:15 96:24 142:3 185:8 190:17 193:1 208:8,24 218:10,12 249:22,24 270:23 271:1 301:18 326:19 327:7 357:12 367:23 368:7 marker 183:12 markers 128:12 177:23 179:16 179:18,19 264:19 319:4 Marketing 1:5 7:9 marking 16:2 367:20 MARTIN 2:8 match 68:9 material 132:23 180:15,16 291:23 296:6	367:4 materials 13:18 15:7,13,14,19 24:6 38:10,13 38:18,21 39:1 183:5 292:8 296:7,9 303:7 316:6 379:11 math 337:21 matrix 362:20 matter 7:8 18:7 22:17 23:19 25:13 232:1,9 307:6 370:4 391:4 matters 371:23 maximum 150:24 162:11 Mayo 5:11 58:20,23 59:5 59:12,19,23 60:3,13,15 61:16 62:11,19 63:14,22 64:5 64:14 65:3,20 66:10,14,23 67:19 68:9 69:5 Mbarrie@bur... 2:9 MC 328:20 MDL 1:9 7:11 22:20 40:4 46:9 MEAGHER 2:20 mean 53:16 54:4 64:9,21 70:4 70:16,18 108:14 112:20 119:13 120:15 121:18 125:16 139:19 167:11 187:5 245:21 262:2 264:9 272:12 277:21 296:1 317:19
---	--	--	---	--

317:20 320:6	177:13 184:11	366:6,9,14	mentioning	220:8,14
335:24 336:11	201:10,17	373:19 374:3,7	324:1	222:15 233:18
377:16	202:2,8 203:15	375:10 381:22	Merritt 6:13	255:3 258:23
meaning 17:16	204:18 205:13	382:15 385:11	271:3,9,19	282:3 283:16
87:10 98:11	205:19 206:14	385:12 388:15	272:4,7 279:5	295:9 365:18
112:23 120:16	206:18 210:19	mechanisms	279:11 281:19	365:20,23
131:3 151:11	210:20 219:2	52:20 91:12	282:4 283:10	366:3,16
169:8 185:1	219:15 220:4	113:24 162:15	283:12	379:10
278:4 319:19	220:20 222:5	167:4 210:7	Merritt's 284:20	methods 87:11
321:18 322:2	225:5,7,11	233:7 247:11	mesothelioma...	141:10,14
means 70:13	226:1,16 227:3	284:15 290:4,7	174:1 176:7	143:19 220:16
80:19 108:16	227:14,18	314:22	meta 224:4	378:23 379:12
133:10 232:17	228:21,24	mechanistic	377:4	microenviron...
308:21 323:15	229:18 230:13	95:23 106:16	meta-analysis	77:18 78:8
meant 131:22	230:13,15,17	232:14 259:24	5:4 23:11	micrograms
measure 212:15	233:20 234:9	262:8	161:2 220:2,19	360:17,18
285:14 317:23	234:20 235:16	mediated	224:17 237:22	361:12
317:24	237:23 238:3	248:15,16	256:15 265:21	micronized
measurement	239:18 242:4	mediator 351:1	376:13	196:12 197:21
318:2 323:1	242:10,20	385:8	metabolomic	microphone
measurements	244:3,15	mediators	319:4	79:14
180:15 285:23	245:13 246:9	238:23	metal 133:11	mid 322:2
measures	247:13,24	medical 97:4	234:18 353:16	mid-'80s 176:1
212:16	249:2 251:24	172:19 310:14	380:22	middle 72:13
mech- 232:14	252:1,5 253:20	meet 108:12	metals 127:14	233:24
mechanism	255:11 257:15	139:3 223:24	127:23 133:7	midway 368:12
20:11 21:6	260:3,7,15	meetings 9:20	133:18 136:9	migrate 162:2
24:2 26:5	261:16 265:16	Melville 2:11	136:21 137:3	165:4 226:17
92:10,14 94:1	266:14,21,23	member 89:13	137:14 146:11	231:13 304:12
94:10 95:12	267:3,9,10,15	98:5 103:13	154:2 352:21	304:16
101:16 107:12	267:20 268:2	members	353:8,12,18	migrates 165:3
113:3 114:4	268:13 275:8	101:12 103:10	381:3,7,15	199:17
116:18,20	277:11 278:9	104:1	382:4	migration 162:6
117:6 126:4,9	278:12 282:11	membership	metastasis	162:7,14
127:24 129:18	284:10 286:18	91:4	366:15	199:22 227:1
135:17 136:3,7	286:19,23	mention 208:6	method 140:23	304:24 305:6,8
137:10 140:18	287:6,10,14,18	385:24	141:18 318:6	milieu 106:8
143:12 145:22	288:1,10 289:4	mentioned	methodologic...	108:2 137:16
146:2 150:20	289:4,8,23	84:12 99:11,14	379:15	235:13 369:8
151:10 155:24	290:9,10,12,15	113:4 115:10	methodology	milliliter 360:17
156:9,15,16,18	290:19 292:4	139:5 148:22	117:3,19	360:18 361:13
156:21 157:1	293:4,11,16	176:8 179:5,14	118:16 121:1	mimic 184:22
157:11,19	294:2,13,17	182:8,22 184:2	140:6,12,13	mimics 191:11
159:13 163:2	295:8 296:1,17	207:10 208:11	141:15 143:2,5	234:11
164:18 167:3,8	315:23 325:2,8	260:1 263:14	143:10,13,15	mind 9:16
167:10,18,23	325:23 326:1	321:22 339:9	143:23 150:13	105:19 147:8
168:23 176:17	353:5 365:5	342:12	188:15 204:7	171:13 184:13

185:11 218:5 228:13 245:1 247:7 285:5 291:17 300:1 301:17 389:18 mindful 158:10 mine 10:17 141:5 371:24 mineralogy 384:1 minerals 383:21 384:4 minimal 162:11 minimum 100:23 150:24 229:24 mining 307:7 minority 127:9 minus 319:6 minute 182:3 minutes 79:21 158:6,7 236:13 236:23 237:3 349:21 356:17 367:16 mismatch 344:9 Misrepresents 53:5 missing 87:5 218:6 261:21 377:9 Misstate 175:18 Misstates 63:16 67:22 117:12 118:21 131:17 132:3 149:22 152:4 153:20 180:6 202:17 205:21 214:22 230:10 248:8 251:22 275:20 296:4 383:15 mistake 332:13 mistaken 342:10 mitochondria 316:9 317:9 mitotic 31:11	188:10 mixture 213:16 MIZGALA 3:9 model 32:8 184:5,21 186:9 186:10 190:2 267:22 268:7 304:9 350:4 376:5 modeling 185:3 models 24:18 120:1 350:10 350:13,16,23 351:2 modest 100:24 modified 41:1 modifiers 343:7 modulate 319:18 molecular 5:17 83:9 84:13 107:2 115:5 177:22 179:16 232:19 285:20 285:21 301:4 328:16 365:4 moment 31:7 45:18 52:3 113:4 129:15 148:23 175:22 208:12 252:17 272:6,11,14 303:24 326:5 369:24 money 300:20 monitor 134:24 monograph 366:22 Monroe 1:16 Montgomery 2:4 month 32:7 months 32:7 Moorman 288:18,22 morning 7:24 8:1 19:2 22:9	23:14 82:21 83:24 185:18 morphologic 328:16 move 304:3 352:2 moved 13:11 311:13 mu- 323:5 mucin-16 183:13 mucin-coding 331:17 332:15 mucinous 328:20 multi-subunit 73:2,11 multiple 358:7 multitude 113:23 mutagenic 77:16 78:6 238:24 355:20 356:2 mutated 217:15 331:2 mutation 53:14 54:2 56:10,17 57:1 64:8,21 242:24 313:8 315:3,6 320:5 320:21 321:2 321:14,17,20 321:23 322:6 322:13 323:5 324:19 325:5 336:11,13,20 339:7,24 340:22 341:19 342:18 345:8 346:17 mutations 51:3 51:13,20 53:10 55:8,14,20 60:10,17,23 260:2 313:17 314:11 320:19	326:2 334:20 335:1,7,10,12 335:18 336:1 338:9,11,21 340:6,8,14 343:9 344:9 346:4 354:21 Myriad 345:20 <hr/> N N 2:1 3:1 4:1 5:1 6:1 109:13 N.W 3:13 name 7:2 8:2,24 50:18 89:6,11 104:22 145:11 307:5 347:17 347:21 351:20 377:13 name's 49:5 named 89:20 308:16 names 127:3 naming 322:22 NAPOLI 2:10 narrative 362:13 narrowing 114:14 narrows 115:13 nation 99:5 national 99:5 108:7,18 109:2 109:15 natural 258:12 nature 8:10 98:11 202:7 234:21 377:23 NCI 108:6,14 109:8,10,12,13 109:17,22,23 110:3,7,11 111:1,4,7 NCI-designated 108:9,9 nearly 61:24 62:10 123:12	329:2 332:18 necessarily 26:12 66:8 103:22 109:12 115:12 163:7 164:6 169:9 174:9 250:20 253:6 258:21 264:21 270:4 270:20 325:24 339:23 necessary 50:16 94:21 114:5 118:11 153:17 155:17 163:18 165:12 171:8 180:11 184:13 222:12 243:14 246:18 252:8 363:22 need 10:23 20:16,21 21:10 27:22 30:6 42:13 69:10 157:1 170:3 172:1 190:14 218:3 237:1 254:2 272:6,11 272:14 276:14 313:13 322:18 379:8,9,9 needed 55:8,14 55:20 95:2 157:14 159:1 needs 162:7 negative 200:13 274:10 312:18 neighborhood 347:23 neighboring 165:7,16 neither 35:23 44:4 312:9 391:11 neoplasia 31:15 187:21 neoplasm 26:1
--	---	--	--	--

30:21 31:3 187:13,16 neoplasms 328:15 neoplastic 25:19 26:11 27:10 30:1 32:3,23 33:2 34:14 35:15 37:5,9 185:18 186:4 190:9,24 363:14 neurological 96:1 never 92:9,13 311:22 new 1:2 2:11,18 2:18,21,21 193:20 318:19 373:5,6 nice 14:4 nickel 366:23 367:8,11 380:17,22 Nicole 84:15 NIH 111:8 nine 98:22 311:9 NJ 2:15 Nods 10:15 11:17 156:23 302:19 non-asbestifor... 197:10 non-finalized 47:1 nonasbestiform 383:22 384:4 nonasbestos 197:20 nongenetic 343:1 nonlitigation 296:15 nonpapillary 31:12 nonprofit 98:9 nonresponsive	269:12 270:15 nonsteroidal 268:21 269:16 normal 62:21 63:3 209:20,23 217:4 218:6 252:21 317:5 317:11 360:14 normalization 212:13 normally 363:11 notably 369:12 Notary 1:21 notation 203:1 notations 333:21 note 88:6 noted 7:13 362:19 392:5 notes 14:5 301:19 notice 1:15 4:14 14:10,21 15:6 15:15,19 23:2 38:8 39:18 62:10 234:9 300:13 notorious 210:5 novel 289:9,19 November 20:2 39:13 41:1,10 42:17 43:9 84:6 85:15 180:2 245:18 NSAID 268:10 268:16 270:8 270:16 NSAIDs 6:14 268:6,9,19,21 269:7,12,16 270:1,11 271:4 NTP 6:3 35:24 181:23 182:16 190:7,20 191:16 192:3 192:12 193:4 193:22 194:7	195:9 198:23 200:8,15 201:2 206:3 207:20 nucleotide 343:4 343:8,15 number 4:13,15 4:17,19,22 5:2 5:6,8,10,12,14 5:16,20,22 6:2 6:4,6,8,10,12 6:16,18 7:11 9:20 14:11 16:4 19:7 21:14 23:3 26:23 30:13 33:7 37:2 38:9 43:12 49:22 59:7 61:17 62:3 66:12,13 66:21,23 67:17 68:8,10 69:21 70:24 72:3 74:10 75:14 81:24 85:19,20 91:8,24 105:21 106:6,11 107:23,23 109:3,8 110:6 115:24 119:23 120:4 121:11 123:5 128:7,9 129:4,5 137:22 142:2 146:19 157:7,13,22 159:9,24,24 168:7,10 174:23 175:11 177:19 186:20 190:16 191:4 192:24 201:9 206:3 208:7 211:9 217:15 218:9 222:23 224:9 227:4,12 227:19 229:24 230:18 234:5 249:21 252:8	263:23 270:22 277:4 279:1,12 279:21 288:3 296:21 310:5 310:21 311:24 312:16,19 313:7 314:3 319:8 326:18 329:21 330:8 347:9 348:1,12 348:20 350:13 357:13 367:3 367:22 368:15 368:16,17 369:9 374:2 376:4 377:17 381:6 384:12 387:2 numbers 161:1 281:15 387:3 numerous 365:19 Nunes 6:17 326:21 327:6 327:10 328:24 330:14 331:9 331:21 333:5 333:11,21 334:1,4,9,18 nutritional 213:6 nutshell 108:22	44:8,19 45:6 46:11,18 48:9 48:15 49:14 52:12 53:3 54:13,22 57:18 57:24 59:2 61:2,8 62:12 63:8,15 65:4 65:22 66:15 67:1,9,21 68:12 69:1,6 70:9 71:7 74:16 76:18,24 77:5,11 78:17 78:23 79:13,19 80:5,12,24 81:20 87:16 88:7 89:15 90:5 91:15,21 92:16 93:7,21 94:13 95:3 97:10 99:16 103:18 104:6 104:13 105:12 107:18 110:15 113:7,18 116:10 117:11 118:3,20 120:8 126:15,21 127:15 128:22 130:10,13,22 131:15 132:2 133:13,19 134:7,19 135:9 136:11 137:4 138:17 140:9 141:4 143:6 144:1,12,18,23 145:18 146:7 146:17 147:16 147:24 148:19 149:4,18,21 150:16 151:18 152:3,16 153:9 153:19 155:3 155:14,20 156:12 157:2
--	--	---	--	--

157:16 158:4	253:12 254:5	356:15 357:2	89:16 90:6	261:14 262:5
158:13 159:4	254:24 255:9	358:1,12 359:9	91:16,22 92:17	263:22 264:24
159:20 160:11	255:19 257:3	360:7 361:17	93:8,22 95:4	266:4 267:1
161:11 162:23	257:20 258:5	363:4 364:19	97:11 99:17	270:3 271:17
164:3,12	258:19 261:6	365:17 367:6	103:19 104:7	275:20 276:13
169:16 170:6	261:13 262:4	367:24 368:5	105:13 107:19	279:10 280:17
170:16 173:6	262:15 263:21	369:21 370:11	110:16 113:19	280:21 281:9
173:14 174:19	264:23 266:3	370:23 371:10	116:11 118:4	282:7 283:20
175:17 177:9	266:24 267:12	373:13,21	118:21 120:9	285:17 287:3
178:7,21 180:4	269:1 270:2	374:4 375:7	126:16 128:23	287:23 289:1
182:5 183:22	271:16 272:10	376:2,17 377:2	130:23 131:16	289:12,17
185:10,21	274:18 275:19	377:14 378:5,9	131:16 135:13	290:21 293:14
188:4 189:1,11	276:12 279:9	379:6,18 380:1	136:12 138:18	294:5,19
190:11 191:1	280:9,16,20	380:11,19	144:2,13	295:19 296:4
191:17 192:13	281:8 282:6	382:11,24	148:20 149:5	296:12,19
193:23 194:19	283:19 285:16	383:14 384:9	150:17 151:19	298:15 299:1
194:23 195:22	286:11 287:2	384:24 385:19	155:4,15	302:21 303:2
197:15 199:9	287:22 288:24	386:7 388:3	161:12 162:24	303:20 304:18
200:16 201:5	289:11,16	389:4,9,12	169:17 170:7	305:2 306:9,17
202:4,16	290:20 291:1,9	390:4	170:17 173:7	314:1 316:21
203:17 205:20	292:5,19	oath 9:17,18	173:15 174:20	318:11 324:24
206:20 207:8	293:13 294:4	10:1 11:1	175:18 177:10	329:5,12,17
208:17 209:4	294:18 295:18	OB-GYN	178:22 180:5,5	332:21 333:2
209:15 210:14	296:3,11,18	257:11	183:23 185:22	333:23 335:15
212:1 213:12	297:14 298:14	obesity 313:15	189:12 190:12	336:4 337:16
214:20 215:9	298:24 302:20	313:21 314:4,7	191:2,18	340:12 341:15
215:18 216:4	303:1,19	314:11,14,18	192:14 199:10	342:3,23 345:4
216:23 217:12	304:17 305:1	object 13:6 21:9	200:17 201:6	345:15 346:20
217:20 220:12	306:8,16	22:3 25:22	202:5 205:21	349:13 350:8
221:6,14 222:1	313:24 316:20	27:21 31:21	209:5 210:15	351:13 352:14
222:21 223:18	318:10 320:12	32:5 34:16	214:21 215:10	354:2,11,23
225:2,16,20	321:9 324:23	36:5,15,24	215:19 216:24	355:12,22
226:20 227:21	325:20 328:3	37:12 43:23	217:21 220:13	373:14,22
228:15 229:8	329:4,11,16	44:14 45:7	225:3,17	374:5 375:8
229:13 230:6,9	332:1,9,20	46:12,19 48:10	226:21 228:16	376:3,18 377:3
231:3,15 232:3	333:1,22	48:16 49:15	229:14 230:7	377:15 378:6
232:11 235:20	334:14 335:14	52:13 54:14	231:4,16	378:10 379:7
236:11,20,24	336:3 337:15	57:19 58:1	235:21 238:17	379:19 380:20
238:16 239:6	340:11 341:2	59:3 61:3,9	239:7 241:21	383:1 384:10
240:11,20	341:14 342:2	63:9 65:5	242:16 243:22	385:1,20 386:8
241:7,20	342:11,22	66:16 67:2,10	245:4 246:24	objected 15:5
242:15 243:21	345:3,14	67:22 68:13	248:8 250:6,10	Objection 53:4
245:3 246:23	346:19 349:12	69:2 70:10	250:22 251:7	54:23 62:13
247:9 248:7	350:7 351:12	71:8 74:17	251:17 252:15	63:16 65:23
250:5,9,21	352:13 354:1	78:18,24 80:6	253:10 255:10	69:7 81:21
251:6,16,20	354:10,22	80:13 81:1	255:20 257:21	94:14 104:14
252:14 253:9	355:11,21	87:17 88:8	258:6,20 261:7	113:8 117:12

126:22 127:16	222:5 269:9	10:23 11:5,15	old 26:20	162:11,19
130:11 132:3	283:3 333:9	11:23 12:2,5	once 8:9 43:21	163:1 164:8,17
133:14,20	369:2,5 376:21	12:19 13:14	oncologist 370:3	164:23 165:2
134:8,20	381:24	14:5,8,20 15:2	oncology 99:2	174:17,21
135:10 137:5	observational	16:14,23 17:2	310:7 323:20	175:5,15
140:10 143:7	375:3	17:15,22 18:12	324:11	178:17,23
144:24 145:19	observations	18:17,21 19:3	ones 263:16	181:17 182:18
146:8,18	116:1 167:20	19:15,23 20:9	ongoing 115:22	183:7 186:23
147:17 148:1	168:20 172:7	21:17 26:13,21	115:24	189:8 190:8
149:19 152:4	210:18 211:8	27:7 30:11	open 251:13	191:13,20
153:10,20	213:3 225:7	35:10 38:1,3,8	operations	192:3 200:20
155:21 156:13	236:1 283:10	38:24 39:6,15	312:6	207:17,23
157:3,17 159:5	284:1,9 323:18	39:24 40:3,22	opin- 93:24	209:11 219:1
159:21 160:12	329:21 376:10	50:3,22 51:11	opine 153:17	223:2,6 224:22
164:4,13 178:8	observed 34:13	60:9 71:23	158:1 165:6	225:10 226:9
188:5 189:2	35:11 99:20	73:20 77:6	opining 184:10	226:14 227:17
195:23 202:17	116:22 183:16	80:21 84:5,10	188:12	228:11,18
203:18 206:21	186:17 197:24	88:1,14,20	opinion 13:16	229:3 230:22
207:9 209:16	224:3 227:8	89:7 93:3,16	26:6,10,14,16	231:20 232:22
213:13 216:5	234:12 258:23	94:9,16,22	27:1,4,6 36:7	235:2 236:1
217:13 221:7	352:22 362:9	95:14 125:19	36:20 38:21	239:14,17
222:2,22	387:13	138:4 141:13	59:4 65:11	241:1,16,24
223:19 227:22	observing 33:1	141:21,24	86:1 93:24	243:18 245:8
229:9 232:4,12	133:1	147:4,9,19	94:18,24 95:8	246:5,20 247:6
240:12,21	obviously 43:6	164:23 167:21	95:17 96:20,22	247:20 250:8
247:10 251:21	177:2 350:2	169:12 170:1,2	102:7 110:8	250:19,23
254:6 255:1	occasion 353:8	193:10 232:16	111:14,16,18	253:5 254:8
257:4 267:13	OCCC 328:20	238:13 240:15	111:23 112:1,5	259:16,19
269:2 286:12	occur 162:6,8	240:24 241:16	112:21 114:12	260:15 261:10
320:13 321:10	164:2 227:1	301:15,22	117:4,9,14	261:15,18,22
325:21 341:3	240:5 344:13	302:16 307:8	118:24 119:3,4	261:24 262:8,8
357:24 359:5	occurred 189:21	307:13,14,20	121:2 122:6	262:13 263:3
360:1 361:7	363:7	307:21 311:18	124:4 126:11	264:6 266:22
363:3 364:17	occurs 227:5	312:24 313:20	126:18,23	267:2 271:22
367:2 369:19	odd 57:16	324:13 325:11	127:12,18	285:19 286:9
370:10,21	311:16	325:15 326:11	128:19 133:4	287:5,7 288:14
371:8 380:12	offer 112:1	326:24 327:17	135:6 136:2,6	290:12 293:22
382:12 388:4	161:6 162:11	327:24 328:13	143:11 145:15	294:22 297:23
objections 15:6	234:19	328:23 330:11	145:22 146:13	298:16 325:4
15:8,11 29:16	offering 52:19	330:14 331:3	147:20 150:19	325:18 351:6
29:19	159:15 266:19	331:11 332:14	151:8,11,21,23	352:5 353:22
obliteration	Oh 29:3 50:2	335:20 339:8	152:6 153:12	354:9,12,17
363:10	175:7 194:24	339:19 342:9	154:8,20	355:19 358:20
observation	301:13 332:10	343:20 348:22	155:22 156:3	363:18,21
31:22 176:20	389:17	351:18 352:3	157:10,19	364:6 365:2
179:7 197:9	okay 8:10 9:9	359:18 360:24	159:15 160:5	367:8 373:18
211:6 212:4,17	10:7,14,19,21	361:18 383:11	161:7,14	387:21 388:1,5

388:20,23	170:4 180:24	104:12,21	254:4 255:15	373:20 374:8
opinions 18:14	198:12 288:19	105:2,10,18,21	256:16,24	374:10,19
20:6 22:20	301:10 302:11	106:23 107:13	257:18 258:3	376:16 377:13
23:18 24:21	311:23 312:5	107:17,24	259:18 260:10	377:18 378:3
25:13 37:3	372:1	110:4,10,13,24	260:12,21,24	378:19 379:16
38:19 39:12	opposed 199:18	111:8,15 112:2	261:5,17,23	380:9,17 381:4
44:24 52:19	opposite 269:23	112:9,22 113:6	262:1,3,14,19	381:12,13,16
65:10 84:6	274:6	113:13,17,22	262:23 263:4,5	ovaries 31:13
85:21 96:23	order 28:2 313:1	114:9,17	263:19 264:5,6	199:18,20
97:3,7 101:24	332:23 333:14	115:18,21	265:18 266:15	226:13 231:14
102:1,12 110:2	333:20	116:18,21	267:16 268:23	253:8 254:22
111:5 112:6	organism 210:8	117:5,18,21	269:8,10,17,20	278:19 281:22
114:21 116:4	organisms 318:9	118:2,13,17	270:10 271:5	284:22 362:10
119:7 120:12	organization	119:19 120:7	271:13,21,23	362:18
121:17 124:2	54:17 58:7	120:24 121:5	273:1,3,5,7,12	ovary 31:14
125:2,13 126:1	organized 13:12	128:4 135:20	273:16,18	32:3 37:18
126:3 127:5	62:3	136:8 162:21	274:5 275:1,13	114:1 116:8
129:13 130:21	organs 165:16	164:9 165:23	275:16 276:3,8	161:18 162:4
130:24 134:14	205:5	166:3 167:4,24	276:10,20,22	162:16,22
136:14,24	orient 47:10	168:3 172:6	277:6,19,21	163:8 164:1,11
148:2 156:8	169:19 170:12	182:12,14	278:6,12,16	164:21,24
169:20 173:3	195:7 272:23	184:12,16	279:8,16,24	165:9,13 166:8
176:13 179:24	359:19	186:12 187:1	282:1,22,24	166:11,12
180:11,12	OSC 328:18	188:14 191:15	284:3,12	187:21 199:24
187:7 193:7	330:7,18	201:10,18	285:12,24	200:6 226:18
195:8 199:2,5	OSE2a 360:21	202:2 203:15	289:6,24 290:5	277:22 278:10
199:16 225:4	outcomes	204:19 206:19	293:19 304:13	285:7,15,24
233:12 244:24	108:21	207:18 208:16	305:21,23	304:16 362:5
247:4 249:1	outside 216:17	209:3,21,23	321:3 322:15	363:12
252:24 262:10	226:17 317:18	211:12 213:22	322:17,20,24	overall 137:18
262:18 275:18	ovar- 282:21	216:20 217:4,7	324:15,16,21	176:21 177:4
282:5 288:10	ovarian 4:24 5:5	217:10,17	325:6 326:3,13	209:17 210:19
291:23 304:23	5:19 6:9,14	218:6,14 219:3	327:1,13 328:2	219:10 235:15
305:12 353:11	20:11 21:7,22	219:8,12	328:13,14	247:13 258:8
365:21 366:24	23:12 24:18	220:21,23	329:1,7 330:24	258:11 270:7
370:1,8,13	25:7,8 26:5,8	221:11,24	331:6 333:5	275:1 281:23
371:5,16,18,22	26:15,17 32:12	222:20 223:11	334:7 336:10	339:22 345:6
374:14 382:21	32:20 36:21	223:17 224:3	337:2,11,20	346:23 366:14
382:21 384:21	83:11 84:14	224:21,24	338:1 340:2	368:20 382:7
386:14,17	89:4,9,14,18	226:19 227:9	341:7 346:5,6	386:1
387:9 389:13	89:24 90:3,8	227:15 228:8	346:18 347:19	overgeneralize
389:21 390:1	90:14,18,21,23	228:12 229:7	349:23 350:6	216:16
opportunity	91:3,9,13 92:6	230:24 237:23	350:18 351:1	overlapped
43:11 47:3	92:11,15,21,23	238:4,10	351:11 360:15	45:16 87:22
85:20 87:23	93:6,20 96:9	243:17 245:15	364:4,7 366:2	overly 312:4
97:14 98:19	99:6,19 100:2	246:7 249:17	366:12 368:21	oversee 99:1
134:11 154:12	101:17 102:8	252:2,12,21	369:10 370:2	oversight 98:17

Overview	page 4:2 5:13	357:5,15,19	301:12 302:18	368:19,22,24
327:13 329:1	18:3 24:8,13	358:5 359:17	302:23 303:7	387:9,10
ovulation 351:3	24:16 30:12	362:12,13	303:17,23	paragraphs
351:7	31:4 33:11,18	367:17 368:11	333:6 334:1	330:5
owned 308:15	35:19 50:20,24	368:14,15,16	340:1 341:5,21	parallel 295:4
oxidation	51:3,7 53:12	368:17 370:19	342:24 359:15	parallels 234:9
315:13	53:19 54:1	372:16,19,20	359:21 361:20	288:5 289:6
oxidative 87:1	55:5,11,12	372:21 373:10	361:23 362:2,6	paraphrase
238:22 316:5	56:15,24 60:3	373:10 374:1	365:5,12	158:1
oxygen 182:1,9	60:6,9,20 62:1	Page/Line 392:6	367:16,17	paren 78:7
209:8 211:4,22	63:1,20,22,24	paid 181:8	368:1,8,12	331:13
212:6 214:12	64:19 71:12,24	295:16	369:5 374:18	parens 73:4,5,13
214:18 218:1	72:1,8 73:8,22	panel 200:24	374:22 378:14	77:16 78:6
236:7 239:22	75:5 76:9 77:9	212:8,8 310:7	378:22 379:12	parentheses
240:19 242:21	78:3 112:10	310:11	379:14	60:16,23 78:16
259:3 263:9	119:6 122:12	Paoletti 124:15	papers 121:21	328:18
286:17 315:10	122:16,18	124:18,22	263:8 283:13	Park 6:19
316:8,11,15,17	123:16 124:15	125:2,8,24	284:8 300:22	338:16,19,20
316:23 317:4,7	124:20 125:15	paper 6:13	331:8 376:20	339:1,21 340:1
317:16,18	137:22 138:7	25:12,16,18	papillary 30:24	341:21 367:16
318:1,3,8,12	138:16 147:7	26:3,10,14,23	paragraph 31:5	367:17 368:1,8
318:16 319:11	149:16 187:12	28:2,10 29:2,2	31:8,10 33:12	part 124:5,8
319:24 359:22	187:17 191:21	29:6,10,23,24	33:14,16,19,24	125:18,24
361:16	193:16 194:14	30:5,6 38:5,5,9	34:1 49:12,13	126:2 130:17
	194:22,22,24	38:17 39:5,11	49:17 51:1,2,6	137:18 145:13
P	195:1,5 196:3	79:8 84:12	51:7,12,17	152:5 157:20
P 2:1,1,4 3:1,1	200:9 201:8,21	87:14 102:19	72:7,13,20	168:22 176:16
392:1	203:2 204:24	126:5,6 141:20	75:23 76:8,20	209:7,7 210:19
P.C 2:3	219:16,20	143:17 168:12	76:23 77:2,9	218:22,24
p.m 166:22	221:9,15,18	177:24 179:21	82:2 112:11	219:7,15
237:10,14	238:1 250:13	180:3,10,20	191:22 203:3	226:22 230:15
302:4,8 356:20	269:13 271:8	181:6,21 184:3	205:1,11	235:15 239:18
356:24 390:11	272:19,21,23	187:24 188:22	221:10,19	266:11 289:13
390:12	274:14,16	220:6,15 224:5	265:15 266:12	289:15 306:19
p13/Ras/Notc...	275:24 278:13	232:22 236:3,6	268:3 269:14	317:5 325:24
330:12	281:20 313:5,8	239:12 267:21	272:20,24	338:3 341:5
p5- 217:14	320:3 326:12	271:3 272:3,5	274:12,15,22	364:6 376:21
p53 216:21	326:24 327:1	276:15 279:2,5	275:24 278:14	381:23
217:4,9,14,18	327:10,12	279:14,21	279:14 314:22	partial 279:13
217:24 218:6	328:4,9,23	282:4,9,12,15	322:5 328:10	328:10
321:22 334:21	330:6 331:20	282:19 283:11	329:1 330:5,15	participants
335:11,17	332:4,7,8,15	283:12,16,22	331:11 332:18	161:1
336:1,16,19	334:13,13	284:1,8,18	333:20 334:19	participate
338:21 339:8	337:1 338:8,8	285:1,20	335:23 340:9	102:3
340:10,19	347:7,8,8	296:23 297:19	343:3,6,21	participated
342:17	348:22 349:19	297:20,24	345:19 348:23	91:1 104:4
packaged 61:17	349:19 350:22	298:4,8 301:9	362:22 368:13	particle 164:11

196:15 249:9 250:4 particles 196:20 198:9,16,17 253:6 particular 24:8 32:9 90:10 143:17 147:22 148:8 151:15 180:17 190:1 191:8 199:6 215:11 216:7 220:24 227:19 232:19,20 235:17 252:9 260:6 276:15 285:5 288:11 322:13 324:20 345:12 346:16 377:21 particularly 26:18 160:24 174:5 210:4 253:15 288:3 301:3 353:18 355:24 381:8 particulates 205:4 206:6 parties 391:12 partner 103:7 partners 102:21 partnership 103:4,6 parts 48:7 352:11 375:20 patent 8:12 9:9 9:21 pathological 351:10 pathologist 370:4 378:24 pathologists 256:24 pathology 25:4 25:7,7 257:5,9 257:13,17 370:2	pathways 185:4 patient 108:19 176:4,24,24 patients 99:22 173:19 175:15 176:4 323:19 344:1,23 345:11 Patricia 288:18 pause 175:22 326:4 pay 144:9 198:20 pediatric 323:10 peer 23:10 299:7 382:22 peer-review 71:2 peer-reviewed 70:19,20,24 71:3 141:17 167:15 288:2 297:3 364:1,10 364:15 366:9 382:18 peers 287:19 pelvic 6:13 271:4,13,21 272:8 274:4 275:10 276:16 276:21 282:20 283:3 284:6 penalty 393:3 Penninkilampi 6:9 218:15 219:17,22 220:6,9 224:5 227:11 236:18 237:18 265:13 267:9 269:24 376:9,13 people 153:1 292:18,18 321:7,8 345:13 percent 101:13 155:1,10 255:17 256:10	256:11 323:12 337:3,4,10,20 343:17 360:13 percentage 152:19 212:6 256:20 percents 322:3 perform 61:20 62:6 134:12 308:5 309:12 performed 99:24 148:4 188:7 189:15 190:1 224:18 386:19 performing 224:12 performs 308:18 309:3 perineal 5:4 6:9 23:11 159:16 162:2 166:9 167:2 177:7 191:11 205:3 218:14 219:12 226:9,12 227:8 227:15,20 228:12,14 230:24 231:5,8 233:22 238:3 253:15 274:23 277:12 278:10 281:21 351:10 376:15 perineally 161:8 161:18 199:8 225:14,23 226:15 231:13 perineum 199:18,23 200:1 229:6 363:12 period 78:10 172:8 173:19 186:18 188:6 224:8 234:6,10 248:19	periods 186:19 224:2 perjury 393:4 person 156:11 259:12 319:14 319:19 336:9 person's 338:11 338:22 340:6 340:14 341:16 personal 3:11 391:7 perspective 71:2 82:6 85:18 115:7 137:9 177:12 184:24 217:23 233:15 234:8 259:24 260:14 295:7 perspectives 57:12 petition 6:5 192:19 195:17 202:9 203:7 372:10 373:3 petitions 202:21 Ph.D 1:14 2:8 7:17 49:21 88:11,16 92:1 392:3,22 393:16 pharmaceutical 299:8 phenomenon 270:12 318:14 318:15 349:16 355:14 phenotype 177:4 252:9 phrase 36:16 330:17 physically 229:24 physiological 314:18,19 317:14 319:7 319:14,21 349:16	physiology 317:5,11 319:17 picking 56:16 picture 209:7 266:16 PID 273:2,4,6 273:12,16,18 piece 27:5 129:16 137:1 229:4 pine 213:11,17 place 17:11 170:14 228:14 248:17 placed 193:12 292:2 placing 185:11 plaintiff 40:4 43:20 48:24 266:1 plaintiffs 8:16 8:18 15:12 16:8 17:3 18:19 19:6,20 23:1 100:15 142:13,22 143:22 144:5 257:1 295:17 plaintiffs' 2:2 12:3,6 17:9,16 17:20 38:12 43:2 54:20 84:23 91:10 93:4,10,17 95:16 96:7,18 102:15 121:15 122:2,4 123:8 123:21 181:9 256:22 288:13 297:10 372:17 385:15 plan 18:18 97:18 platy 128:14 174:11 plausibility 5:23 37:4 52:20
---	---	---	--	---

93:11,19 94:1 94:18,24 95:9 96:4,9,21 111:19 117:7 121:3 127:24 131:1 140:14 142:6 143:19 230:4 288:15 288:23 366:7 385:11 388:20 388:24 plausible 26:4 95:12 101:16 112:8 113:3 114:3 126:3 129:18 135:17 136:3,7 137:9 140:20 143:11 145:22 146:2 150:20 155:24 157:11 163:2 164:17 167:3,8 167:11,23 168:5,23 184:10 201:17 205:3,12 219:2 220:4,20 222:4 225:5,6,11 226:16 227:14 227:18 228:11 228:21 229:18 230:13 257:15 260:20 261:16 267:3 268:2 275:8 278:12 282:11 288:1 289:23 290:4,8 290:14,19 292:3 293:3 294:2,13,17 295:8 296:17 325:23 366:6 375:10 382:15 plausibly 375:10 play 275:13,15 276:2,22 279:7 281:24 282:24	314:19 353:21 playing 388:15 plays 116:24 260:17 264:5 269:19 273:1 276:10 321:2 366:2 please 19:11 25:2 29:15 40:23 52:3 63:21 83:3 182:4 185:13 190:13 221:16 272:15 326:17 360:9 372:9 378:8 pleura 172:20 173:12 pleural 174:2 pleurodesis 129:23 168:13 172:19 173:5 173:20 174:2 174:18,21 175:5,15,24 176:15 177:3,7 177:16 178:5 178:11,18 PLLC 2:10 plural 87:17 plus 235:12,12 235:13 319:6 point 163:9,24 165:21 176:9 183:10 201:9 203:9 227:10 227:24 240:18 266:10 268:10 268:14 271:19 288:2 304:9 319:10 323:11 324:1 343:16 360:15,20 365:1 372:14 374:17 pointed 106:6 194:7 195:8,12	201:21,24 pointing 331:7 points 186:15,17 196:11 197:2 policies 101:22 policy 101:12 poor 319:19 population 337:12 338:1 portfolio 109:6 portion 201:21 374:16 portions 86:14 313:2 posit 257:16 position 98:2 186:3 310:13 positive 200:12 274:10 299:13 335:18 345:21 346:11 371:2 possibilities 108:2 possibility 35:17 97:24 189:18 212:19 246:10 382:2,10 possible 47:12 50:19 69:17 71:9 75:3 89:10 92:24 125:8 145:11 223:13 255:6 336:18 369:6 381:21 possibly 87:19 146:1 231:13 post 173:19 potential 4:23 21:22 83:22 91:12 92:10 93:5 105:2 121:4 137:12 163:9 170:21 214:6 234:19 235:7 237:23 240:9 243:2	245:1 247:20 249:18 250:24 253:16 258:9 259:10 261:11 265:16 266:23 267:8,10,15 297:24 299:16 315:17 344:16 352:21 380:23 381:21 385:9 385:17 387:5 388:13 potentially 110:7 139:6 164:19 176:23 177:4 276:7 353:21 powder 1:5 5:18 6:13 32:19 37:17 83:10 84:14 91:13 92:11,12,20 93:12,19 94:10 94:19,20 95:1 95:7,10 96:5,9 96:15 99:23 100:3 102:7 110:4,9,13 111:8,15,17,19 112:2,8,21 113:16 114:4,8 119:2,19 120:6 120:24 121:4 126:12,19,24 127:3,10,13 128:11,20 130:9,20 133:2 133:12 134:5 134:18 135:1,8 136:8,10,21 137:3,23 138:6 138:10,13,14 139:14,21,22 139:23 140:8 144:10,22 145:7,23 146:1 146:5,14,15	147:6,21 148:4 148:5,11,18 149:2,15 150:2 150:3,3 151:15 151:23 152:1 152:11,11,20 153:5,15,17,22 153:24 154:7 154:13,24 155:9 159:16 161:8,9,17 167:2,22 168:9 177:8 178:2 180:1 181:17 182:13,14,19 183:21 184:11 184:14,15 185:20 186:11 186:24 188:13 193:12 196:21 198:10 199:8 208:16 209:2 211:11,14,18 218:2 219:3,7 222:20 223:10 223:17 225:14 225:23 226:10 226:15,16 229:5 230:22 231:1,8,13 233:7 239:14 239:18 257:19 258:4 261:3,11 271:3 278:10 282:16 290:5 293:18 324:22 352:7,11,18 353:24 358:21 364:14 365:9 366:2 375:4 377:11 384:17 385:6,18 388:19,23 powder-conta... 145:16 Power 7:9 practice 89:24
--	--	---	---	---

90:3,7	259:1 276:20	315:8 323:14	172:19 174:4	products 1:5,6
Practices 1:5	315:7,9 338:9	primary 121:13	procedures	3:11 7:10
7:10	338:11 339:7	174:14 183:4	174:12	127:10,14
pre-reviews	339:24 340:5	188:21 211:5,5	proceeding 40:5	128:16,20
312:1	340:13 341:19	226:8,8 232:21	proceedings	130:9,20 133:3
precancerous	344:19 345:23	305:3	391:4,9	133:12,23
183:21	379:2,16 385:7	Princeton 2:15	process 121:20	134:1,6 137:23
precisely 54:16	385:13 388:12	principle 176:21	171:15 176:15	138:6,8,10,12
85:10	present 125:5	364:1	275:10 295:5	138:15 139:5,7
Precursors 25:8	134:5 136:9	principles	350:20 353:13	139:21 140:8
predated 311:23	162:22 163:8	140:18	353:17	144:11 145:7
predict 323:22	243:9 252:21	printed 19:12	processes 91:24	146:14,16
predis- 331:1	277:16 300:9	83:4	278:1	147:7,21 148:5
predispose	318:13 387:15	printout 14:4	produce 64:14	148:16 150:3,6
107:22 238:9	388:19,22	59:12 60:3	128:12 129:24	151:4,9,24
346:5	presentation	prior 8:22 9:21	157:1 238:8	153:18,22
predisposed	92:13,19 93:1	10:8 17:7 22:8	251:15 261:11	154:15 161:8
323:16	97:15 300:7,9	23:13 45:22	282:16	167:2 177:8
predisposes	presentations	46:8 50:11	produced	178:1 180:1,14
53:15 54:3	90:17 92:22	83:23 91:10	263:10 264:17	193:13 196:21
320:5	presented 97:3	95:15,20 96:7	317:9,17,18	198:1,10,14
predisposing	126:4,9 128:1	142:18 310:13	318:3	199:8 230:23
320:19	129:10 136:4	363:17 364:4	produces 181:17	231:2 234:16
predisposition	225:7 290:11	371:4 372:2	186:24 249:9	243:2 258:4
324:2,3,11	303:7	376:13	250:20 253:7	261:3,11 352:7
325:3,5	presents 245:8	private 98:9	product 7:9	352:11 353:24
preliminary	president 101:8	300:12,16	18:10 44:10	354:7 387:16
319:5	101:20,23	309:24	111:4 138:23	professor
premature	press 89:11	privilege 18:10	138:24 139:14	310:16,19,19
279:3	96:14	44:11 291:4,5	143:21 144:22	310:20,24
premise 52:17	presumably	291:11	145:24 146:5	311:2,6,16
52:22 61:12	145:24	pro-inflamma...	147:12,20	profiles 310:8
165:5 177:14	prevalence	277:5	148:12,18	profiling 99:4
300:3 323:4	260:5	probability	149:2,15 150:4	program 99:9
prepare 13:23	prevention	99:19 321:24	150:11,15	programs
prepared 112:1	193:11	335:2 336:8,22	151:1,1,5,16	109:17,17
preprint 5:17	prevents 217:10	probably 12:8	152:2,12,20	progress 174:24
23:9 84:11	previous 101:2	19:1 43:18	153:4,6,7,16	174:24 205:8
85:5 86:11	163:11 268:3	105:22 121:12	153:22 154:24	207:2 246:19
87:21	273:6 275:5	158:9 177:19	155:9,18 232:2	310:22 312:2
presence 137:7	384:13	198:7 274:12	236:4 291:5	progressed
154:20 198:16	previously	311:9 313:1	352:6,18 353:3	119:22 174:24
200:6 217:23	84:12 351:22	318:14 319:15	353:5 382:7	189:22 206:8
232:19 234:24	primarily 95:21	321:1 363:9	388:14	progression
235:7 239:9,10	121:9 138:8	problems	production 39:2	26:7 32:12
240:1 243:3	156:8 181:2	200:11	98:17 316:8	96:6 117:2,16
249:18 258:24	188:19 211:2	procedure	319:24	118:12 135:19

156:2 169:4	239:9 242:9	289:22 308:19	310:1,2,6	Q
171:9,15	246:10 255:12	322:12 324:18	published 48:8	quant- 139:7
176:19 178:13	284:15 286:19	325:23 341:8	69:21 76:14	quantify 130:6
178:20 179:19	286:23 287:9	352:16 387:17	90:13 92:9	130:17 133:17
204:15 240:5	287:13,17	provided 14:3	96:22 140:23	146:10,15
244:12 245:15	289:8 296:17	23:1,1 39:21	141:10,14,17	147:1 148:14
246:18 255:15	325:2	39:22 40:3,7,9	141:17 143:1,4	156:5 160:6
256:6 260:18	proposition 34:7	42:19 43:1,15	143:5,14,23	161:17 177:5
261:1 262:22	35:8,20 271:11	83:20 84:22	167:14 235:18	231:12
264:19 265:6	273:11 274:3	98:23 122:1,2	265:21 279:5	quantifying
277:18,23	339:15 381:15	123:6,10 129:2	287:9 350:4	139:7
310:10 320:17	propositions	135:23 142:12	354:19 358:7	quantitate
347:24 348:17	339:14	142:24 151:3	358:14,15,22	138:22
363:23 366:15	proprietary	202:14 220:22	364:1,10,15	quantitated
proinflammat...	213:15,16	260:15 290:11	381:2 382:17	166:7
369:8,11	prostaglandins	293:5 296:7	382:22	quantitates
project 91:18	238:24	374:23 384:18	PubMed 121:9	162:18
projects 89:19	protected 291:4	providers 308:7	pull 27:13 74:23	quantitating
91:8 98:21,24	291:5,14 310:3	309:14	124:13 357:9	152:19
99:10 103:24	protein 73:3,12	provides 24:19	357:13 359:13	quantitation
104:11,16,21	216:21 217:4	65:7 98:18	purchased	162:5 179:16
105:1,4 109:3	315:13,16	115:8 220:19	383:13	183:12 231:21
109:19 309:4	343:19	269:17 290:1	purported 257:1	249:4 286:16
proliferate	proteins 329:23	388:14	378:3	quantitative
77:18 78:8	343:12,18	providing	purports 252:11	387:19
proliferating	protocol 196:14	106:12 157:24	380:9 383:12	quantity 387:14
76:16 77:15	protumorigenic	172:13 330:22	purpose 173:12	387:24,24
78:5	349:9	333:7	174:15 211:5	question 9:14
proliferation	provide 15:18	PTI 3:7	213:9 214:9	10:16 20:22
182:10 348:5	36:7 43:17	public 1:21 5:24	219:21 247:23	21:9 25:24
prominent	47:21 84:18	107:11 109:11	362:1	28:3,15,17
321:1	93:11,18,24	109:13,24	purposefully	29:9,12,22
promise 318:23	94:9,17,23	111:7 142:8	172:23	30:2,3,4,7 31:3
promote 239:1	101:9 102:5	300:12,15	purposes 136:24	34:18 36:17
253:8 254:4	108:19 111:16	384:16	199:16	44:17 46:22
promoted	114:24 125:6	publication	pursuant 1:15	47:5 52:4
311:16	140:22 143:11	83:22 97:15,18	put 13:2,10	54:24 61:4
promotes	143:14 145:21	179:12 180:9	37:23 48:14	67:15 70:8,14
243:19	150:23 151:20	279:2 300:7	50:23 59:22	71:16 80:1,14
promoting	152:6 153:11	312:3 333:6	76:8 333:19	80:15 81:12,18
239:5	157:18 159:7	publications	357:9 359:11	105:8 113:9
properties 356:3	161:13 171:16	295:2 296:21	361:19	115:13 116:13
386:24 387:13	222:6,14	300:14,19	putting 344:17	125:16 132:10
proposed 20:11	224:22 228:17	318:23 323:10	373:9	132:12,15
21:6 24:2	228:17 231:20	341:8 365:10	puzzle 137:1	134:23,23
219:14 220:3	254:7,8 261:8	367:4 385:24	229:4	135:1,4,14
233:20,23	285:18 287:24	publicly 309:19		

138:3 145:3	356:12 357:4	rats 187:13	134:21 135:3	68:8 69:16
149:24 150:10	367:14 369:23	192:7 363:5	182:4 272:22	70:1 71:21
152:24 154:4	372:12,13,16	RDR 3:21	273:8 277:14	75:7 84:22
155:23 156:15	380:16 383:3	re- 94:23	288:20 392:3	92:19 100:23
157:7,13,21	386:12,15	reach 164:1	393:4	110:11,23
159:23 163:6	389:3 391:6	166:11 204:8	reading 54:24	111:2,3,12
163:14 164:5	quick 236:16	205:4 224:14	68:8 83:15	123:23 132:24
169:24 170:3,9	quickly 319:18	386:17	132:4,4 194:4	139:16,21
170:13,19	319:21	reached 371:17	194:20 202:18	149:8 152:19
171:1 193:17	quite 45:15	386:14	212:10 221:16	175:23 185:9
197:13 198:4	120:3 184:20	reaching 164:11	294:24	185:14 187:15
201:20 208:21	224:10 263:14	166:8 205:18	reads 31:11 34:1	192:20 193:10
216:1 217:2,19	277:14 299:20	365:21 366:24	51:12,19 53:13	193:15,18
220:10 226:23	301:5 314:15	370:7,13 371:5	54:1 61:16	194:5 208:12
228:2 229:14	319:12,18,20	371:18	77:23 274:22	212:10 249:16
230:18 231:18	319:21 327:18	reaction 31:1	275:24	253:1 257:9,12
232:18 235:22	361:21	205:6 211:22	ready 272:17	275:17 277:6
250:18 251:11	quote 62:20	246:21,22	reality 299:10	285:7 305:5
251:14 252:19	64:19 276:1	250:3 253:17	really 106:21	306:18 322:24
252:22 253:4	304:24	254:23 255:8	109:12 143:17	340:23 342:16
255:23 256:4		257:2,19,24	170:18 300:4	342:19 353:9
262:16 269:6	R	315:1,11 316:8	318:23 382:5	357:16 359:23
270:6,9,9	R 2:1 3:1 391:1	316:14 359:21	realm 297:7	361:22 364:22
273:23 281:2	392:1,1	reactions 250:17	311:1	372:16 378:13
282:2 285:1,4	R.E 25:9	315:4,20	realtime 1:20	380:18 383:4
290:21,22	radiation	reactive 182:1,8	11:11 132:8	recalling 180:23
291:24 292:23	314:23	209:8 211:3,22	135:4,11	receipt 15:10
297:20 305:8	random 320:9	212:6 214:12	391:19	38:12
307:17 314:13	321:7	214:18 218:1	reason 96:12	receive 44:2
319:23 324:14	range 281:15	236:7 239:22	174:14 184:19	45:3
324:17 325:1	ranks 310:23	240:19 242:21	184:19 193:3	received 44:6
335:22 336:17	Rappel@seyf...	259:3 263:9	212:20 254:21	85:6,13 87:20
339:6 342:7,14	3:14	286:17 315:7,9	256:5 361:2	90:20,22 173:4
346:8 353:1	rare 95:23 224:7	316:8,11,15,17	reason- 267:23	302:23 303:5
359:5 360:1	rarity 174:4	316:23 317:4,7	reasonable	receiving 17:7
364:22 377:19	rat 32:7 35:16	317:16,17	171:2 267:24	receptors
377:21 379:22	186:19 189:22	318:1,3,8,12	356:2 390:2	330:20
380:2	190:2,2 304:9	318:16 319:11	reasonably	RECESS 166:19
questions 8:4	362:4 379:20	319:24 359:22	106:17 127:6,6	recognized
10:13 11:11	rate 131:4,13,24	361:16	177:21 214:14	222:14
16:3 29:5	132:19,21	read 31:17	220:2 299:20	recollection
41:18 67:5	133:5,10	53:17 54:5,8	reasons 177:2	22:15
105:5 139:9	285:12 324:11	55:2,9 64:4,5	recall 27:18	record 7:2,14
140:12 156:20	336:19 337:24	65:19 66:22	28:11 30:1,3	11:9 12:13
163:13 292:18	rates 146:20	68:14 74:5,14	38:19,20 43:5	15:4 19:10
300:24 301:22	314:3	74:18 80:2	43:18 45:8,15	23:6 24:12
306:22 307:8	ratios 161:5	113:9 116:12	50:16,17 59:18	25:2 31:10

42:14 82:11,13 82:15 166:13 166:17,21 170:5 202:17 237:9,11,13 272:22 302:3,5 302:7 356:19 356:21,23 390:8,10 records 123:22 312:3 recruitment 316:4 redacted 18:9 redactions 18:6 redirect 42:12 reduce 269:17 reduced 391:7 REES 3:3 refer 17:16 115:2 116:7 135:11 138:12 185:12 190:13 191:21 233:10 243:17 344:7 356:7 refer- 180:24 reference 24:4 25:3,6 34:23 35:1 37:22 38:9,18,20 39:4 73:21,24 74:1 75:2 79:7 112:15 119:6 122:9 125:6 132:22 141:20 143:9 175:11 176:10,12 180:24 181:2 181:23 219:17 266:11 279:23 285:19 298:12 326:15 330:1 339:21 353:7 358:16 385:21 referenced 18:7 24:6 25:11	47:7 84:3 129:3 131:5 132:24 141:10 142:15,17 178:16 180:21 188:18 211:2 214:10 254:18 266:9,9 295:16 300:16,18 303:9,13 333:8 337:7 368:2,9 376:8 references 24:19 24:20 36:6 45:17 79:12 121:24 140:22 145:12 182:8 182:21 183:4 208:5 241:5 325:18 331:10 333:10 341:6 358:7,20 referencing 331:8 referred 302:17 306:6 309:5,7 309:23 330:24 referring 86:21 110:18,20 127:2 150:2 179:21 183:1 190:21 211:21 254:9 264:12 280:2 303:3 305:18 315:5 344:4,5 refers 239:4,8 344:15 reflective 196:14 refresh 30:19 272:14 refute 166:1 204:4 275:12 refutes 210:20 regard 324:18 325:12 342:16	364:4 370:1 regard- 300:11 regarding 4:20 4:23 5:3 20:10 21:5 24:2,16 26:1,16 31:3 37:3 65:13 90:11,14,18 105:5 110:8 111:4 112:16 114:22 115:20 117:14 121:3 126:3,7 127:20 127:21 130:24 132:22 133:23 135:24 138:21 143:23 145:23 148:3,15 154:8 157:10 163:1 165:18 183:7 191:20 204:13 206:5 213:3 233:12 251:23 267:3 268:16 283:3 284:2 285:2 288:10 293:2 299:4 304:24 305:13 319:24 329:7 334:5 339:17 339:18 347:3 360:3 361:23 367:16 370:4 371:12,13 374:2 387:9,19 regardless 325:5 regards 92:20 101:18 284:6 341:6 Registered 1:19 391:19 regular 101:8 regulated 308:10 309:3 rejected 293:12 relate 341:13,16 related 89:9	104:21 119:20 165:18 193:16 199:12 239:19 239:23 240:3 243:11 259:16 268:24 274:13 279:6 285:11 290:7 293:20 314:18 322:20 323:6,6 324:14 339:6 381:21 relates 1:7 93:18 111:8 117:18 134:3 156:6 158:24 167:21 190:4 191:13 192:12 204:14 210:24 217:6 220:10 240:7 250:3 255:4 267:8 268:9 270:1 272:4 329:24 334:6 350:10 353:4 381:13 382:8 387:21 relating 120:23 relation 6:14 249:5 271:4 326:3 361:9 366:23 370:1 371:11,17 relationship 56:9 93:13 100:3 101:14 112:17 130:2 131:2 148:9 161:3 170:21 174:6 183:9 198:15 227:11 248:23 249:6 254:11 271:12 271:20 273:12 273:15,18 274:4,9 298:2 312:13 313:23 314:14 316:13	320:14 324:10 340:2 341:21 386:22 relationships 100:22 147:2 159:10 relative 174:4 186:16 214:15 215:3 244:12 280:1 281:14 321:17 339:22 340:2 341:19 345:12 377:20 relatively 186:21 268:17 338:6 release 316:5,5 released 349:5 relevance 201:2 relevant 119:9 120:11 125:7 125:12 200:21 203:5 210:17 272:3 357:21 reliability 70:7 70:13,17 reliable 70:3,5 71:15 216:10 216:12 reliance 12:24 187:3 relied 75:6 129:12 233:17 291:23 292:2 370:7 rely 44:23 126:5 163:3 178:17 186:22 187:2,2 205:17 208:14 208:24 224:15 228:5 241:18 286:20 288:2 293:1 366:22 370:12 371:16 374:12 386:18 388:9 relying 122:5
--	---	---	---	--

124:1,3,5,9	24:6 25:11	114:3 116:4	368:9 369:17	represents
125:1,4,15,17	27:2 33:1,4,19	119:3,6,16	370:18,19	108:1 259:22
125:23 147:23	34:1 35:3,15	120:12 122:8	371:1,6,11	391:8
176:12 181:16	35:19 36:7	122:13 123:7	372:2,3 375:3	Reproductive
182:17 186:3	37:3 38:6	126:10 127:5	375:12 383:16	83:12 181:6
208:1 235:17	39:12,22 40:23	129:2,3,18	385:4 387:10	reputable
384:20 385:16	41:5,9,24	130:17 131:1,6	387:18 388:8,9	109:11 299:17
386:13	42:16,17,21	132:24 136:4	389:14,22	request 39:2
remain 266:18	43:2,8,9 45:2,5	137:21 138:7	reported 369:12	325:24 372:22
312:19	45:9,11,12,14	139:20 140:5	369:12 376:20	373:2
remained 353:4	45:15,21,22	141:11,20	378:17	requested 93:9
remaining 312:7	46:1,9,10,16	142:16,19,23	reporter 1:20,20	135:16 150:18
remains 55:3	46:22,24 47:2	143:10 147:8	3:22 7:15 10:1	286:5 287:24
60:1 105:11	47:4,9,9,15,16	157:9 160:2	10:12,20 182:2	289:19,21
106:19 258:10	47:17,19 48:1	173:21 180:2	391:19,19	295:1 325:22
265:18 267:10	48:7,14,17,19	180:12,21	reporting 101:1	requesting
267:17 346:23	49:1,7,12,13	182:22 188:8	101:19	193:11 195:12
Remarkably	49:21 50:5,9	188:19 201:16	reports 39:22	requests 14:23
329:15	50:17,20,24	205:13 206:14	40:8,14,15,19	15:5 293:9
remem- 132:11	51:1,6,12,18	208:3,5 214:10	41:11,20 42:15	require 163:8
remember 8:24	52:15 53:13,20	218:19 219:16	43:1,13,15,21	207:12
37:14 184:3	54:1,8,11,12	219:23 220:11	44:23 45:4,20	required 151:16
198:4 237:19	54:17 55:2,6	231:11 232:23	46:6 48:22	153:6 156:18
303:14 324:14	55:12,18,24	244:16,18	49:17 50:10	162:6,12
357:17 372:13	56:1,15,24	245:17 254:11	53:7 54:21	165:13 172:10
386:14	58:11,18,21	257:10,13,16	57:17 58:6	requirement
remind 124:12	59:20 60:1,10	261:3,19	74:12 114:21	100:21,21
338:24	60:20 62:1	264:14 269:13	129:8 130:7	101:1 169:7
reminding 24:5	63:1,12,16,20	269:24 271:9	134:11 135:24	184:18 298:3
339:4	63:23 64:19	275:7 282:5,12	148:15 235:5	300:18
removed 306:14	65:3,10,11	287:10,14,18	245:7 254:13	requirements
RENÉE 3:14	66:13 67:18	288:19 289:3,6	256:23 257:5	100:11,19
renew 301:19	68:11,22,23	289:9 290:1,11	257:10 266:1	research 75:19
repair 64:1,7,9	69:5,15,19	293:9,12,23	273:6 288:3	86:9,9 91:3,18
64:19,21 323:6	71:20,23 72:7	294:3,24	295:3 296:10	91:23 92:5
344:10,18	73:8,23 75:5	295:12 297:8	352:18 375:19	93:4 95:21
repeat 29:12	75:12,18 76:9	299:15 302:24	383:8	97:23 98:6,9
repeating 334:5	77:9 78:4 79:6	303:9 308:6	represent 8:2	98:14,18 99:3
rephrase 134:22	80:4,23 82:22	312:21 313:2,5	307:5	99:7,8 101:15
207:16	84:6 85:15,22	320:3 322:19	representation	105:2 108:3,20
replicated 106:6	86:3,7,14	327:6 330:6	283:9	108:21 115:20
366:16	87:13,21 88:4	332:19 333:3	representatives	115:24 143:3
report 4:16 5:9	88:5 90:11	334:15,17	9:22	143:21 154:11
13:1,22 14:6	95:19 96:24	335:7 341:12	represented	168:11 172:4
15:20 19:23	97:13,19 101:9	346:11 350:21	15:13 27:1	201:15 204:7
20:1,7 22:12	102:11 110:20	357:6 358:6,16	representing 7:3	210:5 284:13
22:17,21 23:21	112:7,10,11	366:5 367:5,18	279:6	298:3 300:4,5

308:11,13,23	238:9 239:11	responsive	85:21 87:4,20	357:20 358:2,6
309:3,4 310:18	240:2,2,16	15:15,19	87:23 91:2	364:24 365:24
318:19 323:9	241:18 242:1,7	270:10	93:11,18 95:6	366:4 367:7
364:10 366:10	242:9,20	rest 47:22	96:2 101:8	371:19 375:9
researched	243:11,12,13	270:17	104:15 105:3	377:5 381:5,19
364:9	244:4,5 245:9	restate 140:11	109:5 116:3	381:23 382:13
researcher	245:10,20,22	176:14 198:6	119:21,21	382:22 383:16
119:11	246:1,1,6,7	208:21 242:19	121:2 122:3,22	386:2 387:6,18
researchers	247:4,18,18,22	259:8 307:19	124:11 128:6	reviewed 23:22
107:11 109:18	248:14,15,18	restating 164:16	134:11 135:23	25:12,15 26:22
researching	248:24 249:3	388:11	138:20 140:2	27:7 38:5
119:16	249:10,15	restricted	140:15 141:19	45:11 50:11,11
reso- 70:22	250:16,20	309:18	142:18 145:9	50:13,15 52:9
resolve 242:1	251:1,15	result 114:17	145:10,14	59:5 83:12,19
resolved 305:9	252:13 253:7	156:16,21	146:19,21,21	120:5,10 130:7
resolves 242:3	253:24 254:17	167:4 242:23	146:24 148:6	132:23 133:12
resource 71:15	255:14,14,21	270:17 313:16	149:6 154:12	133:21 139:16
121:9	258:10,15	315:5 322:16	157:20 159:7,9	142:9 152:9
resources 121:8	259:1,10,12	324:22 335:11	159:22 161:21	160:2,14
121:13	260:13,17	343:11 344:7	166:3 173:22	173:17 174:14
respect 118:24	261:19 264:16	344:12 362:7	175:20,20	193:16,18
respected 288:8	265:9 270:6	391:13	176:2,10	194:11 195:15
respond 291:17	275:3 278:19	resulted 363:9	183:24 192:1	203:5 223:6
291:18	281:22 282:17	resulting 196:13	193:4 198:13	235:4 251:10
responding	284:21 289:5	239:20	203:12 204:2,5	254:10 267:4
212:23	315:2,23 316:2	results 87:11	204:11 207:12	299:23 301:1
response 23:2	316:14 324:13	126:7 129:7	211:19 218:13	338:3 359:2
34:3,12,24	361:3 365:8	148:6 171:18	219:10 220:1	367:5 372:15
35:5,9,10,11	367:13 372:9	171:21 197:6	220:15,18,19	385:23
39:17 92:3	374:18 375:5	213:2 221:4	222:3 223:9,12	reviewer 84:2,4
129:24 154:22	375:24 376:5	231:11 266:16	223:14 224:4	reviewers 83:19
160:10,15,21	376:15 377:12	267:21 275:5	226:8 227:7	reviewing 34:7
160:24 165:8	377:22,23	295:10 308:6	233:16,18	91:2 96:19
165:18,20,22	378:18 379:2	309:13 315:16	245:7,23	132:14 144:8
168:15 170:24	379:17 380:10	315:24 323:5	256:14 263:15	220:9 253:19
171:19 172:24	380:23	324:4 352:24	283:7 289:22	275:17 277:7
173:24 174:13	responses	360:3 361:9,10	290:7,9 293:17	354:5
175:8 178:10	188:24 189:4	373:4	293:21 294:11	reviews 5:24
178:12,18	209:9 240:10	review 5:3 14:18	294:12 299:7	142:7 224:17
181:22 188:20	242:13 244:9	20:14 23:10,10	299:14,18	295:13 296:23
189:8 192:4,18	245:2,13 246:3	25:8 29:6 37:1	301:19 305:15	350:23
197:5,24	246:12 247:8	37:14 43:12	306:19 314:12	revised 46:6
202:12 204:13	264:10	45:10,16 46:20	322:18 330:22	83:21
205:7 206:1,1	responsibilities	48:18 49:6	333:4,7 334:2	revision 47:24
206:2,7 207:1	89:1 98:16	50:13 75:1,16	334:4 341:4,4	rich 77:18 78:9
211:15,16	responsible	75:17 79:6	341:5 351:15	right 11:10 12:3
230:17 233:8,9	169:3 308:13	81:7,14 85:3	355:13,15	12:4 16:18,19

17:4,5,24	313:18 320:11	RMR 391:18	S	141:16 201:9
22:17,18 27:10	327:15 331:2	road 2:10 265:2	S 2:1 3:1,8	202:7 210:10
33:17 35:12	337:8 340:17	348:5	300:17	232:9 293:15
36:2,13 45:22	340:17 341:11	Robinson 1:19	S-transferase	344:22,22
46:10 57:23	351:21 370:19	3:18,21 7:3,15	183:15	says 202:18
61:7 68:3,6	376:24 378:4	391:18	S.W 1:17	221:3 332:14
71:6 74:2,3	378:16 381:4	role 26:17 37:16	Saed 5:21 83:6	349:2 369:5
76:14 86:5,7	382:10	52:19 60:10	83:21 84:16	scale 259:11
88:22 89:5	right-hand	91:23 92:20,22	85:18 86:12,23	312:6
90:15 92:15	272:23	93:5 110:9	123:1 168:11	Scholar 121:10
93:6,20 102:9	rigor 256:8	112:8 117:1,15	177:21 179:12	science 121:10
106:12 113:6	297:4,24	121:5 130:4	180:3 181:4,8	304:24 305:6
117:10 122:10	risk 4:23 5:5,18	135:19 175:9	182:23 183:11	Sciences 83:12
124:13 125:20	6:14 21:22	179:2 191:20	236:2,6 240:18	181:6
130:21 141:22	23:12 83:11	252:2 260:17	241:4,12,19	scientific 70:7,8
142:20 146:16	84:14 110:13	263:11 264:5	247:3 263:8	70:12,17,24
150:10 152:23	110:24 160:3,5	269:20 273:1	285:20,22	71:16 97:5
157:15 159:3	161:4 166:2	275:12,13,16	286:3,6,10,13	109:14 119:11
164:2 165:1	183:16 201:3	276:2,10,22	296:24 298:1,7	120:17 121:16
175:2 183:1	238:4 254:12	277:18,22	299:15 365:6	130:3 143:4
187:13,22	256:16 265:17	278:7 279:7	365:10	144:6,8 148:3
188:24 190:10	267:16 269:17	281:24 282:10	Saed's 87:12	163:17 175:4
194:8,10 195:9	271:5,14 273:4	282:24 284:3	88:4 179:21	175:14 178:15
195:13,15	273:12,16,18	289:5 311:19	180:10 181:13	179:9 185:3
203:16 213:11	274:5 275:1	314:20 321:2	181:15 286:15	187:6 194:16
214:24 215:5	276:20 278:11	326:12 349:22	296:23 297:11	196:6 202:19
221:5 223:7,8	279:16 280:1	350:3 353:21	298:12 300:22	234:22 235:18
228:10 230:5	281:14 282:21	366:1 381:7	302:12,17	241:1 263:2
231:14 240:10	285:3 310:10	388:16	safety 111:4	286:24 287:19
240:19 244:18	313:21 314:14	rolling 320:21	386:24	289:10 295:5
244:22 245:2	321:3,17,19	ROS 215:8,16	Sales 1:5 7:10	297:4,24 299:5
250:14 252:13	322:23 323:2	216:3	samples 99:20	299:10 300:4
258:18 264:10	324:5 334:7	Ross 6:9 218:15	105:6 129:5	373:6 381:2,14
264:14,22	336:15 337:2	roughly 19:2	131:8 308:6	390:2
266:23 267:11	337:10,11,19	98:21 234:10	309:13 371:2,3	scientifically
268:13,24	338:12,22	338:4	383:12,18	70:3,4 136:17
269:21 270:1	339:22 340:2,6	route 199:7,12	384:3,8	216:10
272:5 273:10	340:14,22	routinely 105:6	satisfied 52:9	scientists 89:23
273:13,19	341:7,17,19	RPR 3:21	298:22 299:3	107:11 109:3
276:11 280:2,6	343:1 344:1,24	391:18	saw 20:5 22:16	257:12 288:9
280:7 282:15	345:6,12,23	RS 212:20	27:9 46:4,15	295:9,15 300:9
282:18 283:1	346:13,18	rules 10:7 29:17	47:1,6,10,11	score 249:13,15
287:11,12	347:19,24	29:20 291:22	47:12 90:13	screening 4:20
288:23 295:17	369:10,11	292:20	211:16 385:21	19:14
296:10 302:18	377:17	run 309:24	saying 53:2,6	Scully 3:3 25:9
302:24 303:18	risks 266:15	running 308:6	81:3 107:5	25:12
304:5 308:2,3	273:3 326:2	309:13		search 373:3,5

searches 120:19 121:6	131:12 132:7 137:24 148:7	199:21 200:3 257:10 322:19	344:19 349:2 362:12 369:1	369:10
searching 322:21	159:11 173:5 194:18 195:3	326:22 362:17 363:14	372:21	Serpa 327:7
second 10:3 16:15 18:3	195:24 196:4,9 196:17 197:6	seminal 37:15 senescence	sentences 51:23 52:10 53:21	served 15:6,21 20:1 33:5
33:13,19 41:9 45:21 60:2,6	202:24 203:10 212:9,10,20	212:15 senescent	54:10,15,19 55:24 56:7	services 7:4 308:1,5,12,17
61:15 76:20 112:10 152:24	218:16 221:13 221:20 232:6	212:22 senior 84:16	57:9,17,20 58:3,6,9,14,17	309:6,7,11,12 309:17,21
163:14 187:17 203:3 238:1	238:5,6,11,14 239:2,3 245:1	sense 71:3 77:23 78:11 105:24	63:13 64:4,11 64:13,18 65:2	310:4
253:4 272:18 274:14 278:14	258:2,18 259:7 265:19 266:2,7	116:19 167:19 177:1 189:17	65:19,23 66:4 66:12,13,22,23	serving 8:13 47:9,9
323:13 327:3 328:24 366:11	271:6 276:4,5 276:8 277:5	197:22 277:13 284:9 294:20	67:7,18 68:9 68:10,22 69:5	set 38:18 61:18 122:5 232:17
372:21	278:21 279:18 301:11,17	296:20,21 301:1 315:14	73:17 74:5,6 74:14 75:22,23	334:18 365:7
second-to-last 269:14	306:14 313:9 313:19 325:16	379:1 sensitive 346:15	76:7,10 78:15 78:19,22 80:2	setting 8:9 9:15 10:4
secondary 165:11 323:23	326:6 328:11 331:23 334:22	346:22 sensitivity	80:3,8,16,22 81:5,11,17	SEYFARTH 3:12
324:8	339:5 361:3 368:13,19,21	322:15 324:21 347:3	82:1 263:16 313:12 329:6	shape 319:16 share 197:12
secondly 192:6 section 60:3,14	368:23 369:3 369:14 372:23	sensors 73:1,10 sent 43:21	separate 122:9 131:2 156:17	201:4 shared 24:5 44:5
63:23 79:23 250:14 326:12	373:7,8,10 374:3,6	sentence 33:13 33:15 34:1,4	176:22,22 230:19 233:3,3	44:7 102:11 123:14 179:18
362:22 369:16	seeing 21:13 49:16 111:3,13	51:11,17 52:2 52:14,16 53:1	233:4 243:13 270:9,11	256:22 287:6 308:23
see 9:4 11:7 14:17 19:18	187:15 285:7 299:3 334:1	55:1,5,17 57:7 60:21,21,22	308:15 separated 145:6	SHAW 3:12 Shawn 1:14 7:12
21:10 22:11 23:16 25:23	352:17 371:23 seek 97:18	61:11,15 62:1 62:19 63:2	270:9,11 308:15	7:17 392:3,22 393:16
27:22 29:1,6 31:2 33:15,22	291:13 seeks 290:22	72:13 74:1,18 76:9 77:21,23	separately 245:14 246:7	SHKOLNIK 2:10
34:4 37:24 45:24 50:7	268:15 seemingly 236:6	167:9 196:5 238:2,6,18	246:11 separates	short 97:13 100:3 186:18
51:4,9,15,21 56:21 57:4	seen 14:15 15:20 19:15 22:8	269:5,15 281:19 313:19	144:21 separating	189:16 318:20 shortcoming
60:18 61:22 62:8,23 63:5	23:13 31:12 32:23 50:1,5	239:4 265:16 269:5,15	156:14 247:24 sequencing	200:24 shorthand 391:6
64:11,24 72:10 72:15 73:6	50:19 59:16 74:8 82:21	314:21 327:11 327:18,20	88:21,23 309:18,21	show 25:19 29:4 29:24 37:8
75:1 77:21 78:1,12 86:22	83:14,23 84:2 84:4 113:11,15	328:1,10,24 329:2 330:6,11	310:7 serious 194:17	160:10,21 161:24 184:13
87:22 94:6 110:3,6 112:18	113:21 180:2,8	331:21 340:13	195:21 196:1,7 197:18	190:9,23 212:18 215:7
119:11 122:8 123:3,18			serous 259:21 275:2 328:18	252:6 253:23 324:5 352:23 362:6 367:20

381:7	117:1 161:2	simply 108:16	253:22	389:10,17
showed 129:7,8	179:2 212:18	127:2 130:16	SKADDEN 2:20	sound 16:17
186:4,7 211:3	215:3 228:4	165:4 166:8	Skip 53:20	17:24 75:9
214:16 252:20	253:23 254:2	171:6 177:12	skipping 313:12	311:15
274:3,6 359:21	256:16 280:15	223:24 240:6	slashes 78:15	sounds 16:19
375:15	280:24 281:5,7	248:12 299:6	SLATE 2:20	90:16 131:11
Shower 127:1,1	281:12 324:3	319:15 323:11	slide 300:10	232:8 249:19
127:4,4 138:11	363:22 388:16	356:7	slides 257:17	298:21
138:11 139:6,6	significantly	sing- 112:4	slightly 108:10	source 59:1 70:3
148:12,12	58:8 85:24	single 26:18	115:6,7 265:2	70:19 75:4
150:11,11,14	192:10 274:24	36:11,19 52:14	330:19	124:4 276:17
150:15 151:4,4	signing 46:8	52:16 73:2	slow 182:3	316:10 317:7
178:1,2	signs 214:8	111:13 167:13	small 57:22	317:10
showing 11:11	similar 22:12,13	223:15 228:22	186:21 274:24	sources 65:14
164:20 188:19	57:8,15 62:15	228:22 229:5	363:10	69:21,23 71:1
233:19 236:4	65:16 66:3	229:23 230:5	smaller 154:19	74:10 106:6
239:22 256:16	68:15 74:7,11	252:7 322:20	SNV 343:19	121:12 154:16
263:9 374:18	75:17 78:19	343:4,8,15	SNVs 343:5,11	300:6,11
380:13	79:9,9,12 80:8	374:18,21	soluble 213:16	space 8:22 9:21
shown 34:2,12	80:16 81:5,7	singular 27:5	Somatic 313:8	125:8 174:2
35:4 115:3	81:11,17 82:2	106:15 112:5	somebody	288:4,11
189:4 214:11	82:3,7 85:19	113:6,12,22	320:22 321:19	299:10 308:21
222:24 235:6	96:2 98:6,9	114:16 115:15	321:19 336:11	341:9
306:3 350:4,17	114:21 121:19	115:18 124:3	somebody's	speak 10:9 12:8
360:3 380:23	127:17 128:17	125:5 127:8,19	377:11	40:6 46:13
shows 27:8	130:1 138:20	128:15 129:14	something's	137:2 159:12
36:11 183:20	140:11 143:16	136:15,17	18:2	207:13
207:6 215:15	146:10 154:14	150:4,4 187:3	somewhat 131:2	speaking 109:16
216:2 220:22	204:10 209:24	235:3 282:9	173:8 212:14	167:15 173:22
221:2 300:10	241:3 253:22	286:20	224:7 234:11	256:12 258:8
360:21 375:4	281:14 284:8	singularly 169:3	268:6	259:4 260:23
377:11 379:15	295:9,10,24	209:6	sorry 34:17	278:8 343:13
380:10 381:2	310:11 315:14	sir 88:16 196:20	36:17 39:10	349:3
Shukla 358:13	329:13,15	sister 355:9	40:13 41:8	specie 211:23
358:15 365:11	331:4 333:3	sit 325:19 326:9	50:2 76:1,19	212:6 214:12
sic 75:12	334:12 344:8	355:18	77:1 113:9	species 182:1,9
side 37:23 50:23	345:7 362:18	site 277:21	116:12 134:21	209:8 211:4
50:23 72:18,18	366:18,18	306:1 317:10	134:22 182:13	214:18 218:1
76:8,9 77:8,8	374:15 387:7	sitting 21:2,4	194:20,24	236:7 239:22
298:13	388:6,12	27:18 28:11	195:1 208:21	240:19 242:21
sides 9:23	similarity 79:3	107:15 110:22	214:2 225:19	259:3 263:9
Siegel 327:8	similarly 66:4	149:14 386:4	232:23 272:12	286:17 315:10
sig- 212:18	294:24 295:1	situation 29:3	273:22 331:2	316:9,11,15,17
signal 73:11	simple 184:20	six 324:7	331:23 332:2,7	316:23 317:5,8
348:4	293:8	size 154:17	339:20 351:16	317:17,18
significant	simplistic	196:16,20	354:12 360:5	318:1,3,8,12
32:11 96:18	171:16	198:9,16	361:1 368:16	318:17 319:11

319:24 359:22	71:22 95:24	Square 2:20	80:7 82:4	stating 81:5
361:16	102:2,10	squarely 283:17	108:16 112:6	135:16 186:6,8
specific 25:24	104:22 110:17	St 323:20	113:20 114:2	205:23 206:24
31:2 38:15	111:2 114:13	stack 357:14	118:8 127:5,10	230:11 329:6
62:5 66:18	115:13 128:10	359:14	131:18 132:15	329:19 337:19
89:19 91:17,17	132:14 138:23	staff 308:21	171:6 203:4	345:2
92:12,19,24	147:2 165:23	stages 318:13	213:15 215:1	statistical 99:19
102:24 103:3,5	174:9 176:14	stand 38:23	248:11,12	statistically
104:16 110:19	177:23 181:23	277:14	259:9 274:8	256:12 280:15
115:18 117:22	184:22 186:7	standard 140:13	280:23 305:7	280:23 281:4,7
118:24 121:24	211:22 221:1,3	297:4	319:15 352:4	281:11 343:13
126:5 128:14	225:23 238:18	standards	387:11	343:17,18
133:1 139:2,4	244:2 253:2	186:19	statement 12:12	status 342:18
139:9 141:20	264:11 266:8	stands 74:1	105:16 110:20	STEERING 2:2
144:14 145:1	275:2 285:7	start 8:3 15:24	111:12 112:24	stenographic
145:12 147:8	295:21 304:20	147:19 169:23	113:1 114:12	7:14
147:12 148:7	305:16 314:9	314:17 345:20	127:18 132:12	step 98:7 169:18
159:23 160:22	322:17 336:5	started 9:13	132:22 133:1	174:3 227:2
164:5 166:5	339:6 342:4,24	11:6	162:1 164:15	steps 167:18
172:5 176:3	344:8 350:19	starting 55:19	170:4 202:7	366:8
177:22 178:1	353:2 355:1,15	60:13 195:4	221:22 229:15	stick 305:13,16
179:16 180:13	357:14 380:14	336:7 368:13	229:16 230:12	305:19 306:6
180:14,14	386:10	starts 328:1	235:10 239:12	306:13
182:10 183:9	specificity 347:4	330:7,18	260:11 267:14	sticking 153:3
183:11 197:19	specifics 45:10	331:12,21	269:3 272:1	stop 62:21 63:3
198:22 199:1	314:17	334:19,20	278:23 317:13	358:3
236:3 244:14	spectrum 95:7	348:23	317:15 322:10	stopped 357:21
249:2 257:8,12	98:23 103:23	state 1:21 25:1	327:19 335:16	storage 44:5
270:5 277:10	120:3 137:13	32:10 35:3	341:12,13	stored 86:20
277:18 282:10	151:6	62:2 65:14	343:24 345:16	Stowers 98:10
283:7,8 284:4	speculate 266:5	74:6 107:7	346:9,10	straight 292:20
284:16 286:17	270:16 297:15	109:21 112:15	347:15 348:10	strange 63:7,10
301:6 314:19	speeches 292:17	162:13 174:10	349:8 363:6	Street 1:16 2:3,7
316:3 318:16	spell 331:22	175:12 205:9	371:21 374:7	2:14 3:13
318:22 322:24	spend 47:14,18	247:2 258:14	376:19	strength 224:20
326:2,7 330:23	spent 18:13,22	265:8 267:19	statements	224:23 233:13
334:6 339:17	96:18 361:21	269:13 288:7	110:7 111:3	stress 87:1
344:5 345:17	spoken 286:3	294:10 299:5	169:20 188:9	238:22 317:12
346:15,22	298:10	299:13,19	206:5 226:24	stretching 26:24
353:2 355:14	sponsored 298:3	314:2 321:21	267:24 277:10	strike 57:16
365:8 383:17	sponsorship	329:22 334:3	277:13 283:8	63:7 218:23
388:9	297:2 298:4	347:14 349:11	284:24 285:2	219:5
specifically	299:11	356:2 381:6	378:21	strong 222:24
25:14 30:23	spray 139:24	393:10	states 1:1 55:6	226:11 347:23
35:15 38:11	sprays 139:13	stated 35:13	110:4 272:24	strongest 345:22
43:6 48:21	139:17 145:7	57:11 65:12	359:7 363:8	structure 82:3
50:17 67:3	145:16	66:4 74:11,22	373:1	137:11

struggling 232:6	281:14 283:5	213:5,9,10	202:20	297:5 369:2,6
studied 217:18	285:21 297:2	214:10,14,16	submitting 49:7	suggested 203:9
studies 33:14	297:11 301:5	215:6,12,15,20	subparagraph	359:20
34:2 35:3 37:2	306:3,12 314:5	216:2,7,9,18	64:1	suggestion
37:7 85:19	330:2 334:9	216:20 217:19	subsequent 91:1	58:10 165:17
116:1,5,15	338:3,5 340:5	219:17 220:24	177:18 201:15	358:24
120:1,2,2,2,10	341:11,13,16	221:3 222:10	201:15 224:5	suggests 113:12
128:7,8,9,10	350:24 362:4	222:13,18	352:17	165:19
131:5 132:1	375:13,15	223:15 224:7	subsequently	Suite 2:7,10,14
133:1,12	376:4,6,14,22	233:4,21	297:3 299:7	3:4,8
136:19 137:19	377:4,17,20,21	235:23 239:22	374:24	Suites 1:16
138:22 139:22	377:24 379:20	249:12 250:15	subset 62:5	summarize
140:1,5 145:2	381:7	252:11 253:21	subsidiary	107:4,4
145:6 146:20	study 6:3,7	253:23 273:10	308:16	summarized
146:21 148:22	26:18 27:4	273:17,20,23	substance 57:3	57:14 75:3
149:8,8 152:10	32:14 33:3	274:2,5,11	218:2 291:13	87:10 199:3
157:21 159:9	35:14 36:11,19	275:14 276:6	substances	summarizing
159:22,24	37:13,16,20	277:3 281:19	56:20 154:1	83:18 152:8
160:1,9,14,19	86:10 90:21,22	285:5 286:20	322:9	summary 70:23
160:23,23	133:3 139:7,8	286:20 299:6,8	substantial	140:15,16
161:1,2 162:9	139:8,17,24	299:22 300:2	107:20	143:16 160:23
163:17,21	145:12 152:8	303:13 304:5,8	substantially	164:16 172:11
165:14 166:4,7	152:21 160:15	304:10,14,19	47:6	309:1 320:18
168:7,13	162:17 175:23	319:9 337:7	subtype 260:6	superior 379:15
173:11 175:11	177:22 178:16	340:20 342:19	subtypes 107:2	supervision
179:4,6 180:19	179:14 180:17	350:20 351:21	260:1 273:7	391:8
181:5 184:4,21	183:19 184:6	354:24 355:4	330:24 332:14	supplement
198:20,21	185:17 186:9	363:15 375:3	333:4	25:10 213:11
201:15 204:3	188:7 189:15	375:17 377:10	succinct 65:13	supplemental
206:3 207:14	189:17 190:1,3	378:3,12,17	293:9	38:10,13,18,22
207:21,22	190:7,14,20,23	379:5 380:8,13	succinctly 65:15	supply 338:20
208:1 210:2	191:8,9,11,16	studying 89:24	sufficient	support 26:3
214:11 220:3	191:19 192:12	stuffy 307:11	113:15 114:8	32:1,18 35:24
220:15 222:23	193:4 194:7,12	subject 18:7	114:16 154:21	37:2 38:19,21
224:9 225:8	194:15 195:9	44:10 92:2,4	171:23 228:23	77:20 78:10
226:7 227:5,6	195:13,16,18	127:18 158:11	252:8 320:22	98:21 108:17
228:6,7 230:16	195:21 196:6	158:17 162:9	336:14 344:11	126:8,9 127:23
231:6 233:16	198:23 200:3,9	287:10,14,18	354:4	136:3 166:1
234:1 236:2,9	200:12,15	289:9 294:2	suggest 113:16	175:4,14
241:3,18 245:6	201:2 206:3,12	subjects 119:18	125:17 162:16	176:16 179:10
251:10 253:19	207:5,19,20,20	119:20 200:21	187:5 199:21	180:12 181:17
254:9,18	208:11,14	293:20	205:11 211:20	182:18 186:3
256:15 263:23	209:1,10,14	submission 85:7	216:11 242:3	186:23 187:6
267:5 271:12	210:12,17,18	submit 18:18	244:11 249:11	189:7 191:16
273:7,11,15	210:19,24	submitted 23:9	256:2 260:5	191:19 192:3
274:8 275:6,9	211:1,3,7,10	38:6 83:11	278:24 279:2	194:16 195:8
275:9 279:1	211:13,17	102:14 202:9	283:7 294:22	196:6 202:20

203:8 205:10	36:20 108:19	surrounding	T 391:1,1 392:1	159:17 162:2
205:12 206:14	108:20,23	62:16 119:19	T.C 25:3	162:15,20,21
207:21 209:1	209:10 211:13	133:4 165:16	table 79:17	163:8,10 164:1
211:10 218:21	suppose 262:7	175:9 197:4	tables 384:12	164:10,11,18
218:24 219:6	296:23	268:2 277:10	Taher 38:5 39:8	164:21,24
234:22 235:18	suppressors	362:17	39:11	165:3,5,7,12
240:4 241:1	73:1,10	surveillance	Taher's 38:9	165:15,19,20
243:14 245:12	supracapsular	73:4,13	take 10:20 16:14	166:2,8,10
253:20,20	362:19	survivors	20:14,24 38:1	168:8,13,16
263:2 269:18	sure 20:14,16,23	323:12	45:18 48:13	172:19,20
271:22 276:9	21:2 24:23	susceptibility	59:19,22 71:23	173:5,11 174:2
281:13 286:18	27:3 29:13	321:6	79:24 82:9	174:9,11,17,21
299:6,8 300:10	33:17,18 34:17	susceptible	98:7 115:9	175:5,15,24
338:20 358:20	34:20 39:19	323:7	124:13 158:6,9	176:17 177:2,6
381:14 385:10	40:10 52:4	suspect 222:8	158:11 165:22	177:14,16
385:17 388:15	81:14 83:16	339:21	166:13 167:17	178:5,10,11,17
supported	87:5 101:4	suspected 96:15	175:21 214:24	178:24 179:10
151:10 167:12	102:16 107:1	sustain 76:16	228:14 236:16	183:9 186:5,11
167:13,19,24	107:10 121:11	77:15 78:5	292:24 301:15	187:13 188:1
179:4 206:2	122:16 125:14	swear 7:16	301:16 302:11	188:19 189:9
225:7 239:21	125:20 139:19	sworn 7:19	326:5 356:16	191:11,14,20
242:6 244:3,15	144:19 149:14	sync 316:10	372:8	195:12 196:12
245:8 252:6	173:22 183:1	syndrome	taken 1:15 66:5	196:13 197:11
260:13 261:16	210:9 219:24	343:21 344:1,9	66:10 81:16	197:20,21
261:18 275:9	262:2 272:17	344:23 345:2,5	189:23 391:5	198:1,14,17,23
281:13 284:10	274:2 278:2	345:11,13	takes 170:14	199:3,12,13,17
295:12 300:14	281:2 293:7	synergistic	248:17	199:18 200:5
300:19 366:9	301:13 307:19	369:6	talc 3:2 4:21,24	201:10,18
supporting 5:17	313:3 315:22	synthesis 263:7	5:4 6:9 19:14	202:2 203:15
5:23 83:9	322:22 335:22	355:10 366:5	20:12 21:23	204:14,14,18
84:13 142:6	339:10 344:21	synthesize	23:12 31:1	205:4 206:1,1
183:5 190:8	348:6 368:17	293:21	34:3,11,23	206:6,18
204:3 208:15	382:13	synthesizing	35:4 36:11,21	207:17 209:8
228:7 230:16	surface 31:13	235:24	37:4,8 94:2	212:21 214:13
supportive	surfaces 24:18	system 185:6	99:11,15	214:17 215:7
129:17 168:20	surprise 52:18	186:9,11	101:16 113:2	215:16 216:2
172:13 174:22	52:24 65:17	200:11 214:7	126:8 127:2	218:14 219:12
175:6 176:20	68:18 79:8	216:18 248:16	128:3,14	220:21,23
178:12,19	surprised 66:8	350:24	129:22,22	221:11,23
207:23 210:20	68:21,24 69:4	systematic 5:3	130:3 135:18	222:16,19
219:14 234:19	69:12,13	5:24 23:10	137:10,11	223:7 224:20
244:6 247:13	surprising 63:17	142:7 220:18	144:20 145:23	224:23 227:9
255:15 260:3	197:23	224:17 227:6	150:6,12,21	227:15 228:8
266:19 268:6,7	surrogate	systems 185:2	151:6 154:16	228:12 233:7
268:12 366:14	340:21	210:1 376:5	155:1,10,18,24	233:22 234:3
supports 26:6	surrounded		156:5,11 157:7	235:3,5,12
26:10,14 35:8	96:14	T	157:14 159:1,8	238:3,8 239:9

239:23 240:1,8	139:12,23	183:21 184:11	89:14,20	304:19 347:9
240:10 241:1	talc-induced	184:14,15	technically	371:3 384:3,8
241:16 242:8	26:5 30:21,23	185:20 186:11	311:15	testified 7:21 9:7
245:2,8,18,24	363:9	186:23 188:13	technology	46:15 131:23
246:20 249:8	talc-related	193:12 196:21	32:10	370:6
249:18 250:4	285:6,15,23	198:10 199:8	tell 7:19 8:8	testify 41:19,20
250:16,19,23	talcum 1:5 5:18	208:16 209:2	11:21 21:18	42:3 297:16
251:14 252:1	6:13 7:9 32:19	211:11,14,18	26:13 64:5	testimony 9:16
252:12,20	37:16 83:10	218:2 219:2,7	76:19 83:2	11:13,19 13:20
253:6,15,16,23	84:14 91:13	222:19 223:10	100:17 131:22	26:9 42:14
254:2,17,22	92:11,12,20	223:17 225:14	317:21	53:5 67:23
255:7,13	93:12,19 94:10	225:23 226:9	telling 61:19	71:14,17,18
256:17 257:2	94:19,20,24	226:15,16	62:5	117:13 118:22
257:18 258:15	95:7,10 96:5,9	229:5 230:22	ten 61:10 186:21	131:17 132:3,5
258:24 259:14	96:15 99:22	231:1,8,12	231:17 236:23	149:22 152:4
261:17 263:10	100:3 102:7	233:7 239:14	237:3 387:23	153:20 157:24
265:17 266:15	110:4,9,13	239:18 257:19	tense 57:6,9,22	175:19 180:6
267:15 274:22	111:8,15,17,19	258:4 261:3,11	tenure 311:3,7	184:8 205:22
274:23 277:12	112:1,8,21	271:3 278:10	311:10,12,20	214:22 230:10
277:12,22	113:16 114:4,8	282:16 290:4	311:24 312:1,9	248:9 251:22
278:16,19	119:1,19 120:6	293:18 324:22	312:10	251:23 296:5
279:15,24	120:24 121:3	352:6,11,18	term 138:13	298:23 353:8
281:21 283:17	127:3,10	364:14 365:9	344:6,14	376:12 382:8
283:23 284:12	128:11 133:2	366:1 375:4	terminology	384:13 389:15
284:22 285:2	133:11 136:8,9	377:11 385:6	153:21	389:22 392:3
289:23 303:18	136:21 137:22	talk 147:6,10	terms 70:18,24	393:8
304:12,15	138:6,10,13,14	153:1 193:3	82:2 91:17	testing 99:24
305:4,5 351:11	139:13,21,22	244:18 264:13	117:6 123:8	128:14 129:4
354:20 355:2,3	140:8 144:10	272:19 307:22	128:8 139:17	133:24 148:4
355:6,9,19,23	144:21 145:15	310:12 343:4	146:24 149:10	234:17 235:5
356:1 358:21	145:23,24	343:21 345:19	154:8 159:7,13	308:18 352:23
360:4,5,16	146:4,13,15	350:22	161:4 171:3,3	354:6 370:7,12
361:13 362:4,7	147:6,20 148:4	talked 287:5	181:22 196:15	384:8,12,15
362:24 364:14	148:4,11,17	336:16 341:24	200:4 207:17	tests 309:23
364:21 365:2	149:2,15 150:1	350:12	209:17 220:9	310:5 383:12
365:12 366:10	150:2,3 151:15	talking 41:15	244:12 255:20	384:13
369:12 371:1,3	151:23 152:1	116:6 132:18	255:21 256:1	Texas 2:8 3:4
373:19 374:8,9	152:11,11,20	225:12,13	259:5 278:8	Thank 15:23
374:19,23	153:5,15,17,22	228:10 232:13	305:21 343:7	16:1 40:11
375:15,20	153:24 154:6	257:7 330:16	346:24 360:10	77:12 158:19
376:1,16,24	154:13,24	340:8 350:2	370:24	166:15 302:1
377:6,18 378:3	155:9 159:16	353:13 382:9	terrific 42:22	307:1 359:12
378:18 379:2	161:9,17 167:2	tangential	132:17	361:18 372:5
379:10,16	167:22 168:8	119:24	test 136:17	389:3
380:9 381:24	177:8 179:24	targets 268:19	295:7 309:20	Thanks 390:7
384:1 386:1,6	181:17 182:13	task 290:14	tested 218:3	theoretical
talc-containing	182:14,19	team 89:3,4,13	294:16,20,23	225:12 382:10

theory 32:2 163:23 238:7 238:14 276:9 thereto 391:6 thing 81:4 125:17 295:24 303:11 316:16 316:19 things 57:14 65:14 94:7 102:20 107:2 194:10 195:20 196:11 223:5 227:13 237:21 240:7 266:18 274:16 292:11 300:16 308:7 309:16 313:3 315:8 think 9:13 12:8 37:21 40:17,18 49:9 67:3 69:17 71:19 81:23 90:13 92:24 105:23 106:5,20 107:6 112:3 113:5,22 127:19 130:1 134:16 139:18 154:5 158:17 162:8 166:10 168:7 173:18 174:5,7 175:11 176:19 182:7 184:9,17 191:7 197:1,11,19,22 198:7 201:7 204:10,16,20 206:10,13 208:3 210:16 212:24 214:13 215:20 216:9 216:13,14 222:8 230:1 236:15 244:14 254:2 255:6 256:12,18	259:5,22 260:4 263:23 265:11 266:17 267:23 270:20 272:2 273:20 274:11 274:20 277:8 277:12 278:2 279:4 283:2,5 285:14 290:3 294:8 295:6 296:20 297:3 300:8 301:21 312:12 314:15 323:3 336:6 341:7,17 342:6 342:12,13 351:22 352:4 354:18,18 355:2 356:1,11 365:4 366:17 367:17 368:6 379:15 381:9 385:9,11 390:8 thinking 92:18 149:7 297:17 304:3,4 317:22 338:2 351:14 369:16 third 51:17 72:19 205:1 366:12,13 thorn 154:16 thorns 154:17 154:18,21 thought 46:15 77:1 194:24 274:21 342:9 thousands 323:19 three 40:13 53:21 55:23 58:2,14 158:7 292:11 309:10 threshold 153:5 153:12,15 thresholds 150:24	tie 165:22 174:9 179:6 222:4 tied 177:23 time 7:6 8:23 9:15 10:3 18:22 20:5 22:12,13 27:16 32:10,14 33:2 35:13 48:18 79:5 82:12,15 87:13 88:4 92:5 96:17,19 97:12,14,22 101:3,13 112:6 119:16 130:14 135:11 142:9 158:10 163:9 163:11,15 165:1 166:17 166:21 173:18 186:15,17 188:6 189:15 193:21 202:15 203:9,14 204:20,22 215:21 230:19 237:9,13 245:17 247:16 247:21 248:2,5 248:19 249:8 250:19,23 253:5 255:18 256:8,10,11,19 256:20 259:11 263:14 279:4 292:16 300:23 302:3,7,17,17 311:23 318:21 339:10 356:19 356:23 359:22 360:15,20 361:21 383:2 385:23 390:7 390:10 timeline 189:16 times 2:20 8:8 81:24 162:7	231:17 234:1 280:1 324:7,7 365:19 385:3 timing 101:17 138:24 258:22 tissue 165:7 176:18 249:17 250:17 252:12 252:21 256:24 258:3 304:13 362:20 370:2 376:24 377:5 378:4,19 379:16,22,24 380:10 tissues 189:22 246:4 375:20 title 41:8 89:11 142:5 312:15 titled 60:4 64:1 83:9 103:6 218:14 titles 310:18 today 8:4 11:2 11:13 13:20 14:24 19:16 21:4 27:18 28:11 39:17 43:13 105:11 107:15 110:22 118:13 130:5 149:14 174:5 201:14 205:24 228:3 231:17 244:23 245:11 257:6 263:17 266:20,22 277:11 282:9 315:9 325:9 350:9 357:7,11 365:7,19 367:15 370:6 382:21 384:22 385:3 386:4 389:15,23 Today's 7:5 told 223:5	325:17 tool 88:24 tools 121:7 top 43:17 123:24 326:24 328:11 331:20,23 332:10,15 338:7 347:7,22 topics 96:3 Torre 327:8 total 17:23 18:14 47:18 54:17 totality 26:23 50:14 86:3 119:8,13 120:6 120:23 123:13 126:23 138:9 140:15 167:17 246:2 290:9 352:5 365:24 388:7 touched 168:11 toxicity 203:6 357:21 358:2 359:2 toxicology 195:6 trace 347:10 track 311:3 trade 127:3 traits 345:21 transcript 67:24 391:4,9 392:3 393:5,8 transducers 73:2,11 transduction 348:4 transferred 143:13 transformation 106:9 114:2 115:10 169:5 169:10 171:22 172:14 182:12 189:21 243:15 244:7 344:12
--	--	--	--	--

transformative	378:20 382:23	45:3,22 47:8	351:21 355:3,6	14:21 34:17
246:17	391:9 392:4	51:23 52:10,18	types 91:9 92:7	70:6 80:18
transformed	393:7	53:20 54:10,15	98:24 100:2	93:16 94:22
171:24	TruSight 310:8	54:19,20 57:13	106:18 118:15	117:8 118:12
translated	truth 7:19,19,20	57:17 58:6	144:10 169:13	133:22 144:20
125:10	try 10:9,19 29:5	64:4,11,13,18	212:7 247:7	147:14 149:23
traveling 199:19	125:18 157:24	65:2,14 68:15	259:17 260:9	202:11 229:3
treated 182:13	220:3 256:19	73:17 74:4,14	260:12,21	230:2 232:7
182:14 186:20	268:15 293:11	75:21,23 76:7	277:16 328:17	255:23 283:15
treatment 105:7	313:1,3	78:14,19,21	329:8 330:20	305:20 307:17
129:24 168:15	trying 37:21	79:3 80:2,3,7	330:21 348:12	335:22 344:22
184:24 299:9	38:24 127:6	80:16,21 81:4	typically 311:7	352:22 383:20
tremendously	232:7 283:15	81:7,11,16,24		understanding
281:11	291:12 292:3	83:2 87:18	U	12:14 53:8
tremolite 383:21	292:17 293:7	94:6 147:4,10	U 300:17	107:16 153:14
trial 9:5,7	339:14 349:20	156:20,24	Uh-huh 34:8	169:6,10
224:12 228:1	350:15	157:13 158:7	38:2 59:14,24	307:13 329:24
trials 310:1	tubes 199:24	162:15 163:12	60:12 72:9	understood 10:2
tricky 38:24	304:16 305:22	163:16,16	124:19,21	10:22 105:22
135:3	306:2,5	168:19 173:18	168:17 187:10	106:17 172:3
tried 125:5	TUCKER 3:8	173:21 177:12	187:20 190:22	351:24
200:3 223:12	tumor 73:1,9	178:24 186:15	201:13 213:20	undertaken
trigger 228:24	106:12 183:12	205:1 212:5,7	238:21 259:16	147:1 163:20
trouble 307:12	246:18 264:17	212:24 222:24	271:10 301:24	294:11
troubled 298:11	265:5,9 310:8	235:7 236:9	334:23 349:1	undertaking
298:17	tumor- 369:7	239:19 240:7,9	369:4 381:1	295:9 318:20
true 32:24 34:9	tumor-activated	240:13 244:9	ultimately 17:14	undertook
34:10 35:8,9	264:13	244:22 245:1	umbrella 309:5	366:19
65:3 66:14	tumorigenesis	245:13 246:3	unable 132:18	unfair 189:13
68:11 71:2,12	369:7	247:7,11	160:16,16	unforeseen
74:5,20 78:22	tumors 77:24	270:11 308:20	377:6	363:8
80:23 81:13	78:11 264:9	308:24 309:22	uncertain 238:4	Unfortunately
84:7 90:18,21	273:5 275:3	315:19,19	unclear 265:18	362:24 363:7
96:11,12 104:8	turn 63:20 269:9	317:1,1 329:6	266:18 267:10	unhealthy 314:3
133:6,9 142:16	312:7 335:18	331:17 350:23	267:17 268:13	uninvestigated
143:24 144:3	356:11 357:5	351:2 355:14	uncommonly	165:11
161:10 195:21	357:14 359:17	360:15 361:1	362:19	UNITED 1:1
207:21 213:6	361:19 368:11	type 48:1 137:10	underestimate	universally
216:3 217:11	turned 311:20	144:21 154:6	107:7	169:11
218:7,8 257:16	Turning 73:8	176:22 179:10	undergirding	University 91:6
260:21 271:23	twelve 9:2 76:3	181:18 182:19	365:2	102:21 103:7
300:7 314:9	twenty 234:10	183:7 189:8,9	undergoing	103:10,12,16
317:13,15	two 16:8 17:23	209:13 213:7	256:9	104:4,10,19
338:19 340:19	24:19 35:20	217:3 259:20	undersigned	108:5 310:14
346:3,14 350:3	36:6 40:13,14	259:22 260:8	393:3	unknown
351:9 354:19	40:14,18,18	276:17 316:3	understand	105:11 107:6
374:20 376:1	41:11 43:1	329:20 350:20	10:16 11:1	215:4 336:21

unregulated 106:1	V	vary 259:12 319:6	viruses 313:15	148:13,13
unresolved 279:12,12	vagina 199:23	vast 98:12	Vitonis 338:16	150:23 151:20
unscheduled 355:10	226:17 304:15	123:12 300:13	340:18 341:24	152:5,6 153:11
untested 289:20	vague 130:11	vastly 107:6	342:4,5	157:18 159:2
USB 12:16,19	133:14 145:19	verbalize 10:19	vitro 120:1	161:13,20,20
13:3,9,12,14	148:1 192:14	verify 16:24	128:10 210:2	165:6 208:22
use 5:4,18 6:9	209:16 251:7	75:10 337:21	286:16	228:17 231:19
23:12 59:20	253:13 286:12	version 45:14	vivo 120:1 210:2	254:7 261:8
83:10 84:14	293:14 384:10	46:16 47:11,13	314:10	285:18 287:24
103:3 119:2,19	388:4	61:24 84:12	voice 307:9	312:3 354:3,16
120:6 144:21	valid 286:21	85:4,5,6,8	volume 130:14	380:2 381:5
146:22,22	validate 383:6	86:16,21 87:21	W	387:17
152:12 153:21	383:10	143:15 301:8	W 3:9	water 213:16
159:16 161:3,4	validated 309:20	301:11 302:12	Wacker 3:8	way 9:1 10:12
163:15 166:9	validity 233:12	versions 40:4	wait 182:3 237:2	29:7 31:17
167:2 174:8	233:13	versus 87:21	want 11:8 12:12	63:10 74:7
177:7 182:10	valuable 346:24	136:21 139:23	16:14 29:6	114:14 131:20
191:11 197:20	Vanderbilt 91:5	177:7 244:19	39:1 44:22	236:5 309:1
208:16 210:6	310:14,24	247:4,22	51:1,6 59:22	317:22 331:16
212:13 218:14	311:7,11 312:8	308:11 319:2	67:15 72:12,18	341:1 342:21
219:12 220:23	312:13	360:13	111:24 125:20	364:13 383:23
221:11,23	variability 259:13	viability 212:12	125:23 132:17	Wayne 299:13
223:10,17	variable 139:18	360:10,13,22	135:12 138:5	299:19
224:20,24	139:24 140:4	361:14	145:5 147:5,5	ways 74:11 81:4
226:9,12 227:8	149:9 318:15	video 1:14 7:7	147:10 152:14	116:8 213:1
227:15,20	319:13	videographer	152:24 175:21	we'll 10:9,24
228:8,12,14	variance 343:4	3:18 7:1,3	193:3 216:19	27:16 30:11
230:24 231:1,5	variant 343:15	79:15 82:10,14	230:2 236:18	33:5 37:24
231:8 233:22	343:16 346:13	166:16,20	250:2 264:1	72:1 79:17,24
234:5 238:3	360:14	237:8,12 302:2	272:19 278:2	124:13 190:19
264:2 266:15	variants 343:9	302:6 356:18	292:23 307:22	192:23 236:16
268:4,16,20	variation 258:12	356:22 390:9	313:2 324:17	292:15,24
270:8,16	varies 311:9	view 26:4 59:1	326:15 344:21	339:10 357:9
274:23,23	316:1	115:11 155:2	359:13	we're 28:10
275:4 277:12	variety 108:23	155:11 207:21	wanted 141:19	30:11 42:13
278:3,10	118:14 119:18	208:15 209:2	143:14 315:22	81:3,3 82:15
279:15,24	121:7,10	211:11,13	warning 193:12	118:23 125:21
281:21 299:1	258:14 263:15	218:21 219:6	195:12 203:9	166:21 189:19
374:20 375:4	277:15 284:11	245:18,22	warrant 355:2	201:14 220:6
376:15	286:14 299:12	251:13 260:9	Washington	232:13,13
users 351:11	348:12 352:23	260:19 288:22	3:13	237:13 247:14
369:13	352:24 384:4	289:2,3	wasn't 9:24 86:5	257:6 264:1
uses 70:22	various 333:13	viewed 171:20	111:16 112:23	265:3 267:5
226:14	386:23	299:6	125:4 130:16	277:11,24
		virtually 334:11	134:9 143:8,8	278:3,8 280:12
		virulent 259:21	143:9 146:9	281:19 292:17
				302:7 307:19

329:19 356:23	59:5,12,20	71:20,22 72:1	54:20	102:23 110:18
381:12 390:8	60:14 61:16	72:19,24 73:22	Word(s) 392:6	144:16 185:24
390:10	63:14,22 64:14	73:24 74:12,18	worded 340:13	222:3 243:24
we've 14:22 15:5	65:3 66:10,14	74:22 75:2,3	wording 58:14	287:4 301:16
21:17 22:23	66:24 67:19	withstanding	74:21 79:9	312:17 313:20
29:13 48:2	68:9	206:12	82:3 274:7	321:5 329:18
49:1 50:8	websites 74:12	witness 7:16,18	283:8 329:10	wounds 77:24
58:17 59:10,23	week 291:22	8:13 12:11	329:13 330:19	78:11
67:3,3,17	weeks 14:19	100:14 102:4	334:11,12	write 48:4 55:13
68:14,14 74:8	weighing 119:8	141:6 158:16	wordings 66:18	58:16 73:9
75:11 79:4,20	weight 283:12	158:20 170:8	words 48:13,21	78:4 150:19
81:23,23 86:16	weighting 284:7	185:12 225:18	56:12 58:4	278:13
87:14 104:16	284:17	237:5 285:22	61:10,10 62:10	writes 56:17
104:16,20	WEIL 2:14,17	291:11 391:10	317:1 330:12	60:15 72:24
127:2 135:22	welcome 42:12	392:3 393:1	work 18:9 44:10	76:15 77:14
158:5 175:8	166:24 237:16	witnesses 52:18	48:24 51:14,20	383:7
180:24 189:19	302:10	57:14 223:2	53:10 88:20,24	writing 47:17,19
205:14,15,24	well-established	247:3	92:1 98:19,24	58:21 69:14,18
206:15 208:3,4	116:20,23	woman 161:7	99:13 100:14	71:20 95:19
208:24 209:6	117:5 130:3	164:9 226:14	103:15,21	142:19 391:7
218:12 226:5	167:12 168:1	322:13 324:19	104:4 106:11	written 39:14
227:4 236:12	353:19	346:16	108:24 120:19	41:3 54:15
242:6 249:24	well-evidenced	woman's 304:15	131:11 158:1	64:13 96:20
253:19 262:18	112:7 115:4	340:21	181:4,13,15	142:23 187:23
263:8 264:3	well-known	women 199:7	183:6,11,14	203:21 357:17
267:18 271:1	183:12 217:14	205:7 207:2	224:6 236:17	wrong 204:20
279:14 282:8	well-powered	225:13,22	244:9 245:14	272:5 303:23
291:21 293:22	228:1	257:17 258:4	284:11,12	wrote 64:16
295:2,3 296:21	well-supported	306:14 336:9	286:9,13,16	85:15 86:6
296:24 301:18	251:24	338:5 346:3	291:5 297:1,5	87:13 88:5
304:10 323:18	went 67:12	369:11 380:9	299:18 326:6	193:11 195:11
323:20 325:9	116:3 200:23	women's 206:8	worked 104:10	245:17
327:7 330:15	324:15	227:10	104:20	Wu 338:16
336:6 348:14	Werb 5:15 75:7	wondering 47:1	working 11:24	342:1,16,17,24
350:9 353:17	75:13 79:8	110:22	14:3 79:16	
355:23 363:20	Whatever's	Woodford 184:2	109:4 115:11	X
370:15 374:15	237:3	Woodruff 184:2	298:13	X 4:1 5:1 6:1
377:16,16	wholly 308:15	207:4,20	works 29:7	232:8,9 387:24
379:20 385:3	wide 32:14	303:14,22	workshop 201:1	
388:6	106:18 108:23	word 68:10,10	world 71:11	Y
weak 304:24	162:8 184:20	78:14,21 80:2	98:20 289:10	Y 232:8 387:24
305:6	284:10 286:14	80:10,21 81:9	377:11	yeah 21:12 22:4
Web 121:10	widely 102:12	81:13,19 103:4	worldwide	79:2 118:23
web-based	Wikipedia 5:13	264:2 278:3	327:4	140:11 141:7
121:8	69:14,18,24	299:2 331:17	worries 332:13	157:9 170:18
website 5:11	70:2,20,22	332:23 392:6	worthy 125:11	181:2 190:15
19:12 58:21,24	71:3,5,12,15	word-for-word	wouldn't 85:11	207:11 231:19

237:6 241:14	270:14,19	357:5 358:5	1st 359:7	42:23 181:15
241:15 248:10	275:24 321:14	142 5:22	<hr/> 2 <hr/>	301:2
296:20 298:19	343:17 360:9	14th 42:17		2018 16:9 18:24
328:6,6 334:17	360:10,12	15 6:2 19:2 99:5	2 4:15 33:6,7	20:2,6 39:13
335:16 343:8	361:9	158:6 190:16	48:2,4,14 49:2	41:1,9,10
350:12,14	1.01 280:7 281:2	190:19 203:6	60:2 96:24	42:17 43:8,10
370:17 378:11	1.17 280:1	319:6 337:4	97:4,8,20	45:24 46:16
380:7	1.2 281:15	349:21 370:19	112:10 119:6	84:6 85:13,15
year 85:2 101:2	1.36 280:7	1508 250:13	137:22 195:1	87:14 88:4
101:5 310:5	1/8/19 4:18	1510 3:4	268:5,18	179:21 180:2,3
311:12 357:22	10 5:12 72:2,3	16 4:17 6:4 75:2	269:11 270:14	180:10,20
years 9:2 26:19	75:22,24	192:23,24	270:19 313:7	181:16 204:12
27:1 98:22	154:24 348:22	357:13 372:8	321:2,12,14,17	245:18 265:22
120:4 161:23	10:15 82:12	16-2738 1:7	327:10,12	302:18 333:6
223:23 227:19	10:25 82:16	16th 16:17	328:24 331:20	2019 1:11 7:5
234:11 299:19	100 255:17	17 2:14 6:6	332:4,8 339:7	16:10 107:15
309:9 310:21	256:10,11	137:22 138:7	341:21 345:7	393:10
311:6 312:16	10036 2:21	138:16 147:7	346:13	208 6:6
319:6	10153-0119 2:18	149:17 208:7	2:10 237:10	21 4:22 6:16
Yep 328:7	103 31:4 187:12	208:10,18	2:26 237:14	326:18,21
yesterday	187:17 362:12	209:1 359:14	20 6:12 47:20	327:8
288:20	105 362:13	170 310:8,8	50:24 51:3	218 2:3 6:8
York 2:11,18,18	11 1:11 5:14	174 272:19,23	53:12 55:5	22 6:18 124:15
2:21,21	75:14 76:4,6	274:14 278:13	56:15 101:13	124:20 367:21
younger 319:17	349:19	175 281:20	236:13 270:22	367:22
<hr/> Z <hr/>	11:51 166:18	18 6:8 122:12,18	271:2 337:4	220 24:14
Zelikoff 5:9 49:4	11747 2:11	123:16 218:9	200 338:4	23 5:2 24:13,16
49:21 50:10	11th 7:5	218:13	20004-1454 3:13	233 3:8
56:16	12 5:16 75:12,24	18-milligram-...	2002 90:23	24-hour 360:14
Zelikoff's 49:7	76:2 82:19,23	192:9	2006 328:15	249 6:10
49:13 50:18,24	84:10,11 86:17	1800s 116:22	2007 181:20	25-fold 337:12
51:1,18 53:13	87:3,15 181:6	19 4:19 6:10	2008 203:7	337:22
54:8,12 55:2,6	269:13 271:8	249:21 250:1	279:5 357:22	26 5:21
55:18,24 56:15	12:52 166:22	190 6:2	358:3,8,22	26th 83:5
68:22	1200 98:19	192 6:4	359:8 372:16	270 6:12
Zena 75:7	125 179:20	1971 37:13	373:4	2738 1:9 7:11
zero 360:17	183:13	184:3 304:4,8	2009 358:15,17	28 41:1,10
<hr/> 0 <hr/>	12th 18:23	1979 303:22	365:11	2nd 16:9 41:7
08542-3792 2:15	13 5:20 79:21	1980 203:7	201 2:14	42:23
<hr/> 1 <hr/>	82:19,23 83:4	359:8 372:15	2011 272:3	<hr/> 3 <hr/>
1 4:13 14:9,11	83:23 84:3,5,8	373:11	2014 6:5 192:18	3 4:17 16:4,7
14:22 39:18	219:16,20	1984 25:5	193:21 194:11	60:9,20 62:1
193:16 268:5	324:7	1993 181:23	202:1 204:9,17	63:1 194:14,22
268:18 269:11	14 4:13 5:22	194:8 201:2	357:17 359:7	194:22,24
	33:11,19 35:19	1994 201:1	373:4,11	195:5 196:3
	142:1,2 143:21	1995 25:10	2017 16:17,22	212:2,3 213:3
	144:6 219:16	1996 249:12	17:7 41:7	

214:24 331:20	54:1 55:12	8		
332:16 360:3	56:24 204:24	8 5:8 49:20,22		
361:10	313:5,8 326:12	50:9 338:8		
3:33 302:3	326:24	367:17 368:11		
3:48 302:8	5,000 98:21	368:14		
30 5:6 26:19	99:10	8-fold 337:13		
30- 338:5	5/2/18 4:18	80s 190:1		
305 2:10	5:20 356:24	816 3:4		
307 4:5	50 155:10	82 5:16,20		
326 6:16	323:12	850 1:16		
33 4:15 17:23	500 2:7 310:11	8th 16:10		
18:14	347:23,23			
357 4:6	360:17 361:11	9		
36104 2:4	361:12	9 5:10 59:7,11		
367 6:18	56 371:1	59:23 60:13		
37 371:1	581 359:17	75:5 76:9 77:9		
372 4:7	59 5:10	78:3 347:8		
389 4:8		9:04 1:18 7:6		
3A 212:5	6	90 322:2		
3B 212:5	6 5:2 22:24 23:3	900 2:7		
	23:7,13,16,18	90s 176:1		
	23:23 24:1,15	96 250:1		
	38:4 191:21	975 3:13		
4	328:4,9,11			
4 2:20 4:19 19:4	330:6 372:20			
19:7 20:4,9	373:10 390:11			
21:5 22:14	6:00 390:12			
55:11 63:20,24	60 319:19			
64:19 200:9	60606-9997 3:9			
201:8,9 203:2	6950 3:8			
357:15,19				
372:16 373:10	7			
374:1,2	7 4:4 5:6 30:12			
4:54 356:20	30:13 71:24			
40 161:23 337:3	72:8 73:8			
337:10,20	185:9 194:14			
40,000 338:5	334:13 337:1			
400 2:10	361:20 372:20			
41 371:2	7-337:13			
42 221:9,15,18	72 5:12			
371:3	72-hour 360:11			
45 238:1	360:15,20			
49 5:8	74 278:13			
	75 5:14			
5	75202 2:8			
5 4:22 21:14,18	767 2:17			
21:24 22:8,11	78701 3:4			
22:16,19 50:20				
51:7 53:19				